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ELECTROPHYSIOLOGY OF THE VISUAL SYSTEM

A SYMPOSIUM HELD AT WILSON HALL, NATIONAL INSTITUTES OF HEALTH,
BETHESDA, MARYLAND
JANUARY 16 AND 17, 1958

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M. G. F. FUORTES, *Editor*

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SYMPOSIUM

on the

Electrophysiology of the Visual System

held at

Bethesda, Maryland

January 16 and 17, 1958

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SYMPOSIUM

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Electrophysiology of the Visual System

Participants

Armington, John C.
Department of Experimental Psychology
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Bennett, Michael
Department of Neurology
Columbia University
630 West 168th Street
New York 32, New York

Bishop, George H.
Department of Neuropsychiatry
Washington University School of Medicine
640 South Kingshighway
Saint Louis 10, Missouri

Breinin, Goodwin M.
Department of Ophthalmology
New York University
Bellevue Medical Center
550 First Avenue
New York 16, New York

Brown, Kenneth T.
Wilmer Institute
The Johns Hopkins Medical School
Baltimore 5, Maryland

Crampton, George H.
Department of Experimental Psychology
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Dodt, Eberhard
William G. Kerckhoff-Institut
Bad Nauheim, Western Germany

Forbes, Alexander
Biological Laboratories
Harvard University
Divinity Avenue
Cambridge 38, Massachusetts

Frank, Karl
Laboratory of Neurophysiology
National Institute of Neurological
Diseases and Blindness
National Institutes of Health
Bethesda 14, Maryland

Fuortes, M. G. F.
Ophthalmology Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health
Bethesda 14, Maryland

Goodman, George
Manhattan Eye, Ear and Throat Hospital
210 East 64th Street
New York 21, New York

Gouras, Peter
Ophthalmology Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health
Bethesda 14, Maryland

Grundfest, Harry
Department of Neurology
Columbia University
630 West 168th Street
New York 32, New York

Gunkel, Ralph D.
Ophthalmology Branch
National Institute of Neurological
Diseases and Blindness
National Institutes of Health
Bethesda 14, Maryland

Hartline, H. K.
Rockefeller Institute for Medical Research
66th Street and York Avenue
New York 21, New York

Hubel, David H.
Department of Neurophysiology
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington 12, D.C.

Jacobson, Jerry H.
Department of Electrophysiology
New York Eye and Ear Infirmary
Albert Einstein College of Medicine
New York, New York

Kennedy, Donald
Department of Zoology
Syracuse University
Syracuse 10, New York

KUFFLER, S. W.
Wilmer Institute
The Johns Hopkins Medical School
Baltimore 5, Maryland

LINKSZ, ARTHUR
Postgraduate School of Medicine
Manhattan Eye, Ear and Throat Hospital
New York University
New York, New York

LIPETZ, LEO E.
Department of Ophthalmology
Starling-Loving Hospital
The Ohio State University
Columbus, Ohio

LIVINGSTON, ROBERT B.
Director of Basic Research
National Institute of Mental Health
National Institute of Neurological Diseases and Blindness
National Institutes of Health
Bethesda 14, Maryland

MACNICHOL, EDWARD F., JR.
Department of Biophysics
The Johns Hopkins University
Baltimore 18, Maryland

MARSHALL, WADE
Laboratory of Neurophysiology
National Institute of Mental Health
National Institutes of Health
Bethesda 14, Maryland

RATLIFF, FLOYD
The Rockefeller Institute for Medical Research
66th Street and York Avenue
New York 21, New York

RUSHTON, W. A. H.
Trinity College
Cambridge, England

SJÖSTRAND, F. S.
Karolinska Institutet
Stockholm, Sweden

SLOAN, LOUISE L.
Wilmer Institute
Johns Hopkins Medical School
Baltimore 5, Maryland

SVAETICHIN, GUNNAR
Department of Anatomy
School of Medicine
University of California
Los Angeles 24, California

TASAKI, ICHIJI
Laboratory of Neurophysiology
National Institute of Neurological Diseases and Blindness
National Institutes of Health
Bethesda 14, Maryland

WAGNER, H. G.
Naval Medical Research Institute
Naval Medical Center
Bethesda 14, Maryland

WALD, GEORGE
The Biological Laboratories
Harvard University
Divinity Avenue
Cambridge 38, Massachusetts

WIESEL, TORSTEN
Wilmer Institute
The Johns Hopkins Medical School
Baltimore 5, Maryland

WOLBARSHT, MYRON L.
Department of Biology
The Johns Hopkins University
Baltimore 18, Maryland

YEANDLE, STEPHEN
Department of Biophysics
Johns Hopkins University
Baltimore 18, Maryland

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RESPONSE PATHWAYS TO ELECTRIC STIMULATION IN THE LIMULUS EYE*

LEO E. LIPETZ, PH.D.
Baltimore, Maryland

In 1952 Hartline, Coulter, and Wagner[1] reported that passage of an electric current through the whole lateral eye of Limulus elicited a discharge of impulses along the optic nerve. This discharge could be detected from single active fibers of the nerve. I later suggested[2] that this response was initiated through electric stimulation of either one or both of two distinct mechanisms, called the "early response" and "late response mechanism. The results implied that the lateral eye has two types of structure which, when electrically stimulated, could initiate independently the discharge of impulses along a single optic nerve fiber.

In this paper are reported experiments aimed at identifying those two structures.

A lateral eye and several centimeters of attached optic nerve were excised from an adult Limulus. The eye was mounted (fig. 1) with its cornea contacting one electrically isolated pool (of seawater) and its back contacting a second electrically isolated pool (of defibrinated Limulus blood). A stimulating electric current was passed through the whole eye from one pool to the other. The optic nerve was led into a third electrically isolated pool (of blood), and a bundle of fibers containing only a single active nerve fiber was dissected free from the nerve trunk and lifted onto a recording electrode. The other recording electrode was placed in the third pool. The passage of nerve impulses along that single active fiber was the response observed on electric stimulation of the whole eye. A detailed description of the experimental procedure is available elsewhere.[2]

The anatomy of the Limulus lateral eye has recently been redescribed.[3-7] However, the structure of the eye is still not completely known. The known structures which are directly involved in the experiments reported here will be briefly described.

The light receptor organ of the lateral eye is the ommatidium. In each eye are about 700 ommatidia, lying just behind a transparent, faceted cornea. Each ommatidium is nearly cone-shaped and consists of a number of different cells (fig. 2). The broad end of the ommatidium abuts the cornea (1) and encloses a cone-shaped chitinous lens (3), which projects inward from the cornea. The outer layer (2) of the ommatidium is made up of pigmented epithelial cells. En-

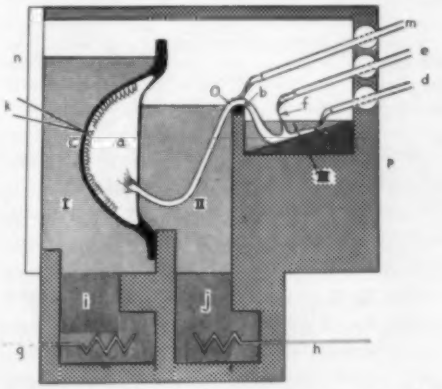

Fig. 1 (Lipetz). Experimental arrangement for electric stimulation of the whole Limulus lateral eye. *a*, the eye; *c*, corneal surface of the eye; *o*, optic nerve; *f*, bundle of optic nerve fibers containing only a single active fiber; *e*, *d*, Ag/AgCl wick type recording electrodes; *m*, grounding electrode; *g*, *h*, large Ag/AgCl stimulating electrodes; *i*, *j*, seawater agar bridges; *I*, corneal stimulating pool, seawater; *II*, stimulating pool at the back of the eye, defibrinated blood; *III*, recording pool, defibrinated blood; *p*, plexiglass moisture-tight chamber; *n*, glass window; *k*, beam of light directed so as to stimulate only the ommatidium from which the active fiber originates; *b*, electrically insulating barrier between pools II and III. (From Lipetz, L.: Dual response of the Limulus lateral eye to electric stimulation. Am. J. Ophth., **44**:118-124 (Oct. Pt. II) 1957.)

* From the Jenkins Laboratory of Biophysics, The Johns Hopkins University.

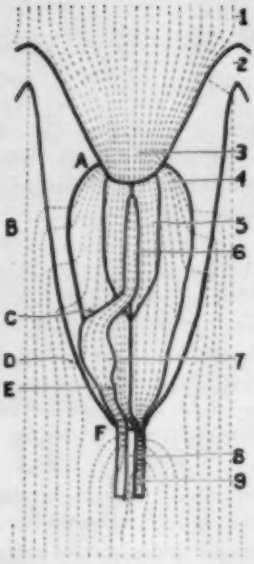

Fig. 2 (Lipetz). Schematic representation of the stimulating current path through an ommatidium on electric stimulation of the whole Limulus lateral eye. See the text for an explanation.

closed within is an ellipsoid-shaped grouping of eight to 20 elongated, wedge-shaped cells (4) termed retinula cells. They are aligned side by side, parallel to and encircling the central axis of the ommatidium, much like the segments of an orange. Along its axial border each retinula cell has a thin, specialized structure, the rhabdomere, which extends centrifugally about one third of the distance along each of the two axial margins of the cell. It resembles in geometry a narrow strip of cloth placed with its long midline against the narrow edge of an orange segment and then folded flat against the sides of the segment. Miller[7] has found that the ultrastructure of the rhabdomere has certain similarities to that of the outer segments of vertebrate rods and cones. The rhabdomeres of adjoining retinula cells lie against each other along the folded flat portions. All of the rhabdomeres together form a structure called the rhabdom (5) which in cross section is like the hub and spokes of a wheel.

Situated to one side of the central axis near the proximal end of the ommatidium is the body of the eccentric cell (7) (usually one, occasionally two, per ommatidium). This cell has a distal process (6) which lies at the central axis of the rhabdom and extends almost to the chitinous cone. A fiber (8) emerges from the proximal end of the eccentric cell, and, similarly, a fiber (9) emerges from each of the retinula cells. All these fibers run to the back of the eye and converge with the fibers from other ommatidia to form the optic nerve. Just behind the ommatidia they pass through a plexus of nerve fibers interconnecting the ommatidia.

On the basis of physiologic experiments, Hartline and his collaborators[3,5] and Waterman and Wiersma[4] concluded that the discharge of impulses recorded in a single fiber of the optic nerve apparently originates in the eccentric cell of the ommatidium from which that fiber comes. Waterman and Wiersma also obtained evidence that the axons of the eccentric cells are the active fibers, and that these axons are the only fibers in the optic nerve which are capable of conducting nerve impulses, even under direct electric stimulation of the nerve. If this is true, then the nerve impulse responses to electric stimulation of the whole Limulus lateral eye originate either in the eccentric cell axon from which the response was detected or in the body of the eccentric cell.

Results and Discussion

When the lateral eye is stimulated as described above, the electric current could conceivably act preferentially at the following regions: (1) site of emergence of the optic nerve from the pool at the back of the eye (o of Figure 1), (2) the back of the eye, (3) the nerve plexus in the lateral eye, (4) within the ommatidium.

Consideration of the geometry of current flow through the optic nerve permits prediction that stimulation would occur at the site of emergence from the pool at the back of

the eye only for current flow with the cornea made anodal, and would always result in responses of shorter latency than for cornea-cathodal current. The observed responses and latencies were in such major disagreement with the predictions that it is unlikely that any of the responses originated from stimulation of the optic nerve where it emerged from the stimulating pool at the back of the eye.

Except just after molting, the back of the lateral eye is covered with a plate of chitin. Several bundles of fibers emerge from the eye through tiny holes in the plate, and converge to form the optic nerve. The possibility was considered that the chitinous plate caused the stimulating current to be concentrated through the holes, and therefore to be dense enough to stimulate the nerve fibers at the holes. That this was not true was shown by the findings: (a) that the electrical resistance of the stimulating current pathway through the preparation was unchanged by removal of the plate; (b) that the responses to electric stimulation were not significantly different in eight preparations from which the plate had been removed.

It is difficult to conceive of any other site along the optic nerve on which the stimulating current could act preferentially. Therefore only the eye itself is left as the probable region within which the electric stimulation occurred.

From the known rate of conduction of impulses along the optic nerve, of two meters per second,[8] the minimum observed latencies of the responses, and the measured location of the distal recording electrode, it can be computed that the nerve impulses originated either within or not more than half a centimeter behind the eye. This again points to the eye itself as the most probable region of origin of the responses.

Of the 31 single active fiber preparations which showed a normal response to light and normal inhibition of that response during passage of cornea-anodal current through the eye, every one of them was able to display both "early" and "late responses." Six of the ommatidia from which these fibers originated were not selected in any way. The remainder were selected in only one way—if the axis of the ommatidium was markedly off the perpendicular to the corneal surface, the ommatidium was rejected for use in these experiments. The ommatidia rejected were all located at the extreme margin of the eye. However, the six unselected ommatidia were all marginal ones, and they showed the same behavior as the more central ommatidia. Since the ommatidia were not selected in such a way as to insure their giving both "early" and "late responses" to whole eye electric stimulation, and yet all of them gave both responses, it seems highly likely that every normal ommatidium is capable of giving both responses. As a corollary, it is likely that associated with every normal ommatidium there is a pair of structures one of which initiates the "early response" and the other the "late response."

It is possible that one or both of these structures is located in the nerve plexus behind the ommatidium. However, the nerve plexus is unlikely to be a site of stimulation because the plexus fibers have a relatively small diameter, which generally is accompanied by a high threshold. The nerve plexus is not definitely ruled out as the location of such structures, but evidence presented below makes the ommatidium itself a more likely location.

As previously stated, the known structures capable of producing the nerve impulses which were observed in response to electric stimulation of the whole lateral eye are the axon and the body of the eccentric cell.[3-5]. Since there are two types of responses ("early" and "late") and two known structures capable of producing such a discharge of impulses along the active optic nerve fiber, it is of interest to see whether each of the two responses can be linked to one of the two structures. This will be done by comparing the characteristics of (1) the "early response" and (2) the "late response"

with those of responses known to originate from a neuronal axon or a cell body, respectively. Corroboratory evidence is obtained by identifying the ommatidial structures in which the direction and relative intensity of the stimulating current flow could account for excitation of each of the two types of response. All the work was done with electric stimuli of no more than three times the value of the threshold strength-duration product.

The chronaxie for the "early response" varied from preparation to preparation in the range from one to four msec.[9] These values are in agreement with the range of one to four msec. found by Monnier and Dubuisson[9] for the chronaxie of Limulus fifth appendage nerve.

On repetitive stimulation of the whole eye (two preparations) with sufficiently strong cornea-negative two msec. current pulses, an "early response" occurred for each pulse at all frequencies of stimulation up to about 60/sec. As the stimulation frequency was increased, an occasional stimulus failed to elicit a response, and this occurred more and more often until a response was elicited by only every second stimulus, and at still higher frequencies by only every third. Similarly, direct electric stimulation of the optic nerve of one of the above preparations produced in a single active nerve fiber a nerve impulse in response to each current pulse, of either polarity, at all frequencies up to about 65/sec. At higher frequencies, the optic nerve showed the same dropping out of responses as was described above for the whole eye preparation. The maximum frequency in response to illumination of a single ommatidium was higher, about 100/sec.

The threshold for the "early response" was measured at various times following application of a subthreshold electric stimulus to the whole eye. The results (shown as curve A of Figure 3) indicate that the threshold dropped greatly (excitability increased) during the subthreshold stimulation. Following the stimulation the threshold (and excitability) rapidly returned to normal, being nearly fully recovered in less than 10 msec. Hodgkin has shown that a single axon of crab nerve undergoes in about four msec. a very similar change and recovery of membrane potential at the cathode following a brief electrical stimulus,[10] and that this change in potential has the same time course as the change in electrical excitability of the nerve.[11] So it can be concluded that the change in excitability of the "early response" following a subthreshold electric stimulus is similar to that of a crab nerve axon.*

These similarities of the "early response" and an axonal response lead to the expectation that the "early response" originates from direct electric stimulation of some portion of the eccentric cell axon.

The chronaxie of the "late response" varied with the preparation from 150 to 600 msec. This is 150 times the chronaxie of Limulus fifth appendage nerve.[9]

The change in threshold of the "late response" following application of a subthreshold electric stimulus to the whole eye was measured, and the results shown as curve B of Figure 3. The recovery to normal excitability is very much slower than that of the "early response" (curve A) and of crab nerve.[10]

The recovery of the "late response" excitability is approximately exponential, with successive half-times of 200 to 280 msec. The only measurements found in the literature to which these data could be compared was in some work on the cat by Eccles.[12] He measured the synaptic potential set up in sympathetic ganglion cells on stimulation of a curare-blocked synapse with a preganglionic volley of nerve impulses. This potential rose to a maximum in 10 to 20 msec. and then decayed slowly. There was summation of the synaptic potentials set up by two pre-

* It should be pointed out that Limulus and crab, while both marine arthropods, are not closely related.

ganglionic volleys; and if the summed potential was high enough, the postsynaptic cell discharged impulses. Thus, the synaptic potential was a measure of the excitability of the synaptic region of the postsynaptic ganglion cell. The decay of the synaptic potential was approximately exponential, with successive half times of 40 to 60 msec. This recovery to normal excitability is more rapid than that of the "late response"; but this is just the kind of difference that would be expected in comparing processes in a warm-blooded animal (the cat) with those in a coldblooded animal (Limulus).

From these findings it can be concluded: (a) it is highly unlikely that the "late response" is produced by direct electric stimulation of the eccentric cell axon; (b) the "late response" may possibly be produced by stimulation of a postsynaptic region of the eccentric cell, either directly or via a synaptic structure.

Recently evidence has been presented[13-17] that in the spinal motoneuron of the cat all

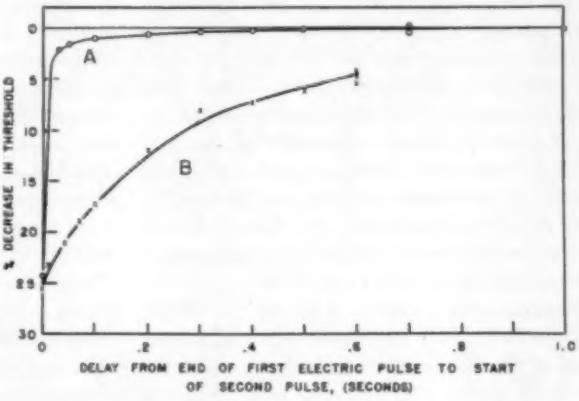

Fig. 3 (Lipetz). The change in thresholds for the "early response" and "late response" at various times after a subthreshold electric stimulus. The conditioning electric pulse and the test pulse were both of the same duration and intensity. Their intensity was adjusted so that a threshold single nerve impulse response was obtained to the test pulse, but not to the conditioning pulse. The percent reduction in threshold intensity of the test pulse from that without a conditioning pulse is plotted versus the delay between pulses. Curve A is for the "early response" to 3.0 msec. current pulses; curve B is for the "late response" to 100 msec. current pulses.

These curves are not a true measure of the change in excitability because the conditioning pulse was not held constant in intensity. It is known from other measurements that the durations and intensities used were within the range in which the product of the intensity times the duration of the stimulating pulse remained constant for a threshold response. So it is reasonable to assume that the amount of response is proportional to the product of intensity times duration of the pulse; that is, the excitability change produced by a given amount of intensity times duration adds linearly to that produced by an additional amount. As the interval between conditioning pulse and test pulse was decreased, the intensities of the two pulses were lowered equally to keep the test pulse just threshold. On the basis of the previous assumption, this reduction in intensity gave equal reductions in the excitability changes produced by each of the pulses. Therefore, the test pulse would have remained just threshold if the intensity of the conditioning pulse had been held constant and the intensity of the test pulse reduced twice as much. A check on the assumption was made by determining the threshold intensity for a single pulse of twice the duration used for the conditioning and test pulses. Its threshold intensity was lower than that for the test pulse alone by very nearly the 25 percent predicted by the assumption. (The threshold changes found are plotted on the curves as the points for zero delay between the conditioning and test pulses.) Therefore, if the values of the ordinates are doubled, the curves shown above will be a true representation of the changes in excitability following a threshold electric stimulus.

For longer delays between electric pulses than those of the last points of curves A and B it was not possible to get a response to the test pulse alone, though a response to the equal conditioning pulse alone was really obtained. This constitutes qualitative evidence of a refractoriness raised threshold) existing at the time of application of the test pulse as a result of the action of the conditioning pulse. This retractoriness was evident for delays of from one to at least six seconds for the "late response," and there were partial indications of a lesser refractoriness over this delay range for the "early response." It is significant that a similar sequence of excitatory and then refractory periods is observed following a light flash. The relation between the excitability changes following a light stimulus and those following an electric stimulus will be considered in a later publication.

nerve impulses normally arise in the region of the axon hillock. It may be that the eccentric cell behaves similarly, and is caused to generate nerve impulses more readily by depolarization of the axon hillock than by depolarization of any other region of the cell. In this case the "late response" might be expected to arise at the axon hillock of the eccentric cell as a result of either direct electric depolarization of the hillock by the stimulating current, or by indirect depolarization of the hillock via local action currents from another region of the cell which was itself depolarized either directly by the stimulating current or indirectly via a synapsing structure.

When the ommatidia were probed with a micropipet electrode (for technique see reference 3), it was observed that the electrical noise level increased markedly if, and only if, the micropipet's tip was inserted in a pigmented epithelial cell. This indicates that the pigmented cells have a higher electrical resistivity than any of the other ommatidial cells. The total resistance of the electrical circuit passing from a micropipet electrode to a remote electrode placed in the seawater covering the eye was measured (for method see reference 18, p. 109). The measured resistance was, respectively, eight, 300, and 10 megohms when the pipet was inserted in the seawater, in a pigmented cell, and in other ommatidial cells. The resistance of such a circuit is determined primarily by the resistivity of the material at the extremely small tip of the pipet. The pipet used was filled with 2*M* KCl solution. On the assumption that the resistance-determining volume of surrounding fluid or tissue just outside the pipet's tip was equal to the resistance-determining volume of KCl solution just inside the tip, the resistivity of the pigmented cells was calculated to be over 1,000 ohm-cm., while that of the other ommatidial cells was about 28 ohm-cm., and the seawater was 20 ohm-cm. This ratio of 40:1 between the resistivities of the outer and inner cells of the ommatidium means that the electric current will flow primarily through the inner cells (retinula and eccentric cells) of the ommatidium, rather than through the outer (pigmented) cells.

The electric impedance across a mounted eye (alive or dead) was found to be a pure resistance of 1,000 ohms. From this and from approximate measurements of the cornea's surface area and thickness it was calculated that the average resistivity of the cornea was about the same as that of seawater, 20 ohm-cm. Therefore, the cornea could have little effect on the path of the stimulating current.

Figure 2 is a diagrammatic representation of the stimulating current flow predicted by physical theory from the above relative resistivities of the ommatidial cells. It takes into account that the ommatidia whose responses were used in the whole eye electric stimulation experiments were selected to have their axes perpendicular to the cornea and to the back surface of the eye. The broken lines represent typical current paths.

This diagram of the stimulating current flow should not be considered a quantitative description, since it is based on a very limited knowledge of the electric impedance of the Limulus eye. For such a description one would need detailed measurements of the geometrical configuration and size and the electric impedance of every portion of all ommatidial and other eye structures. Alternatively, the current flow could be measured more directly with point by point mapping of the potential field which the flow sets up within the ommatidia and the rest of the eye.

The significance of the diagram is that it shows qualitatively how the high resistivity of the pigment cells causes current concentrations. One concentration is at the tip of the chitinous cone, region A. Another is at the neck of the ommatidium, region F. It is in such regions of concentrated current that electrical stimulation is most likely to occur.

When the corneal electrode is made posi-

tive, the current flow is from the cornea to the back of the eye. (From top to bottom in the diagram.) When the corneal electrode is negative, the current flow is the reverse.

The "early response" to cornea-positive current flow can be explained in accordance with the postulates of the previous sections by the heavy outward current through the membrane of the eccentric cell axon at region F. Such an outward current is in the right direction to depolarize the membrane and cause it to fire impulses.

The "early response" to cornea-negative current flow is not as readily explained. At region E there is some current flow outward to the retinula cells from the first portion of the eccentric cell axon and from the axon hillock of the eccentric cell. It may be that these specialized sections of the neuron are sufficiently sensitive for this smaller current to initiate there the discharge of nerve impulses.

The retinula cell fibers experience a concentrated outward current flow at region F during cornea-positive stimulation. But it is difficult to conceive of any site at which they experience outward current flow during cornea-negative stimulation. This is an additional reason for considering the retinula cell fibers to be unlikely site of origin of the "early response" which occurred during both directions of stimulation.

The "late response" occurred during cornea-negative stimulation only. During such stimulation there is concentrated outward current flow at region A from both the retinula cells and the distal process of the eccentric cell. There is also a less concentrated current from the eccentric cell body to the retinula cells, as shown at region C. In addition, during cornea-positive stimulation there is some current flow out of the retinula cells, as shown at region B, and out of the eccentric cell body, as shown at region D. However, since these currents must pass through the high resistance of the pigment cells, the currents are likely to be too small to cause stimulation. Therefore, the eccentric cell body, the distal process, and the retinula cells all can be considered to have current distributions such as would be expected of the site of action of the "late response" mechanism. The action would be a depolarization of any or all of those structures. Such a depolarization could conceivably spread synaptically from the retinula cells to the distal process; from there depolarization could spread to the eccentric cell body and to the specialized region of the eccentric cell at which depolarization causes the generation of nerve impulses.

It has recently been reported that upon thrusting a micropipet electrode into an exposed, living eccentric cell in an opened ommatidium, large action potential spikes were observed, and from no other structure in the ommatidium could such spikes be recorded.[5] As part of this study, these observations were confirmed in over 20 such preparations. In some of the eccentric cells during illumination of the ommatidium there were detected both the positive-going action potential spikes and a slow, positive-going change (termed the ommatidial action potential) in the membrane potential. Changing the depth of the pipet's penetration into the eccentric cell, or withdrawing the pipet and reinserting it in another portion of the cell was found to change reproducibly the relative amplitudes of the spikes and the ommatidal action potential elicited by a constant light stimulus. This can be considered evidence that the ommatidial action potential and the spikes originate at separate regions, and, together with evidence obtained by MacNichol[6] and by Tomita,[19,20] supports the idea that the ommatidial action potential is transmitted proximally along the distal process to a region of the eccentric cell at which nerve impulses are generated. It therefore seems likely that any or all of the three sites mentioned in the preceding paragraph could be the site at which the stimulating current initiates a depolarization which then spreads to the appropriate region (possibly the axon hillock) of the eccentric cell and there initi-

ates the generation of the "late response" nerve impulses.

Summary

In a previous paper it was reported that passage of an electric current through a whole Limulus lateral eye resulted in the discharge of nerve impulses along a single active optic nerve-fiber preparation by either or both of two distinct mechanisms, called the "early" and the "late" mechanisms. Other workers have shown the great likelihood that such a nerve impulse discharge can arise only in the eccentric cell or in the eccentric cell axon of the ommatidium from which the active fiber originates.

The present paper deals with the sites of the two electric response mechanisms. Evidence is presented which is consistent with the ideas that:

1. Associated with every normal ommatidium is a pair of structures, at one of which the stimualting current initiates the "early response," and at the other, the "late response."

2. It is highly likely that the responses originate within the eye.

3. The "early," but not the "late response," resembles an axonal response in its chronaxie, its maximum repetition rate, and the change in threshold following a subthreshold electric stimulus.

4. The change in the "late response's" excitability following a subthreshold electric stimulus resembles that of the synaptic region of a ganglion cell.

Measurements were made of the electric resistivity of the cornea and the ommatidial cells. On the basis of these and of estimated relative cross-sections of the cells and their fibers, current concentrations were predicted in the ommatidium at the chitinous cone and at the neck. The resultant current distributions do not readily explain the "early response" during cornea-negative stimulation. They are consistent with the possibilities that:

1. The "early response" during cornea-positive stimulation is caused by depolarization of the eccentric cell axon.

2. The "late response" is caused by depolarization of the eccentric cell body, of the distal process of the eccentric cell, or of the retinula cells; the depolarization conceivably spreading synaptically from the retinula cells to the distal process and then to the specialized region (possibly the axon hillock) of the eccentric cell at which it causes the generation of nerve impulses.

Acknowledgment

I wish to thank Dr. H. K. Hartline and Dr. E. F. MacNichol, Jr., for allowing me the use of their laboratories, and for their very helpful criticism of this report. Robert J. Rubin assisted with the micropipet experiments. This work was aided by a fellowship from the National Foundation for Infantile Paralysis, by a grant from the Ohio Lions Eye Research Fund, and by Graduate Medical Training Grant 2B-5008 from the National Institute of Neurological Diseases and Blindness, Public Health Service.

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Discussion

Fuortes: Dr. Tasaki would like to start the discussion of this paper.

Tasaki: In the tactile endings in the skin and also in the auditory endings in the ear, the polarity of the electric current effective to initiate nerve impulses is opposite to that in the Limulus eye (late discharge). This means that the electrode on the nerve endings has to be connected to the source of current and the electrode near the nerve fiber to the sink. This fact is in good agreement with the theory that adequate stimuli are eventually transformed into electric stimuli for the sensory nerve fiber. The structure of the sensory endings in the skin or in the ear is simpler than that of the Limulus eye. The effect of electric currents upon the Limulus eye appears to me far more complicated than what you have postulated. This is just an expression of my skepticism.

Fuortes: Thank you very much, Dr. Tasaki. I suppose that I could now take advantage of my privileged position and show a few of my own slides. I have been interested in the same problem which Dr. Lipetz has just discussed but I have approached it through a somewhat different technique which I had learned from Dr. Frank and which we had previously applied to the study of the properties of spinal motoneurones. A small electrode is introduced in cells of the ommatidium of the Limulus eye and is used both for recording the potentials developing across the membrane following illumination, and for passing depolarizing or hyperpolarizing currents. Large spikes can be recorded from elements of the Limulus ommatidia only in the minority of successful penetrations. However, I shall discuss only some of the features of these units producing large spikes because I think that these can be identified with the eccentric cells.

The first slide (fig. 4) illustrates one of the points mentioned by Dr. Lipetz, showing that a sustained depolarization follows light stimulation. The frequency of the trains of spikes superimposed to the sustained depolarization are an approximately linear function of the log of light intensity, as previously reported by MacNichol.

The second slide (fig. 5) shows the effect exerted upon the same unit by depolarizing currents through the microelectrode. It is seen that depolarizing currents of one-second duration evoke repetitive firing and that frequency of firing increases with in-

creasing current intensity. It should be noted that the use of a Wheatstone bridge prevents recording of a sustained depolarization evoked by the current. The results illustrated by these two slides are in general agreement with those presented by Dr. Lipetz, as well as with those previously reported by others.

The finding shown in the third slide (fig. 6), however, differs considerably from those described by Dr. Lipetz. When electric stimulation is applied through the microelectrode (which I presume to be situated in the soma of the eccentric cell) one finds that latency decreases as intensity of stimulating current is increased following a single smooth curve such as illustrated. The solid line is the theoretic curve representing the relation $I_{rh}/I = 1-e^{-\frac{t}{\tau}}$ and it is seen that the experimental points fit this curve reasonably well. In this, as well as in other cells, the time constant τ was of the order of 10 msec.

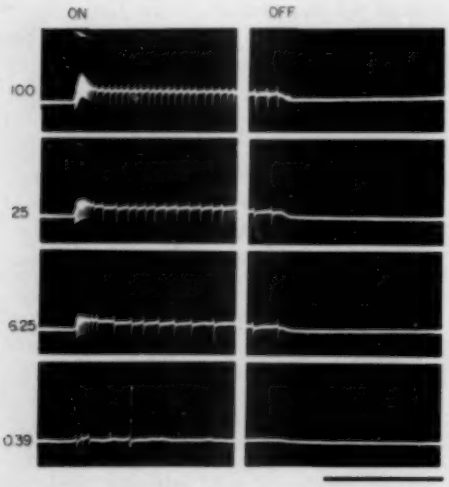

Fig. 4* (Lipetz). Responses of a cell in the eye of Limulus to light. Figures at left give relative light intensity. Ten seconds elapse between end of *on* record and start of corresponding *off* record. Time line: 1.0 sec.

* Figures 4 through 7 were presented by Dr. Fuortes.

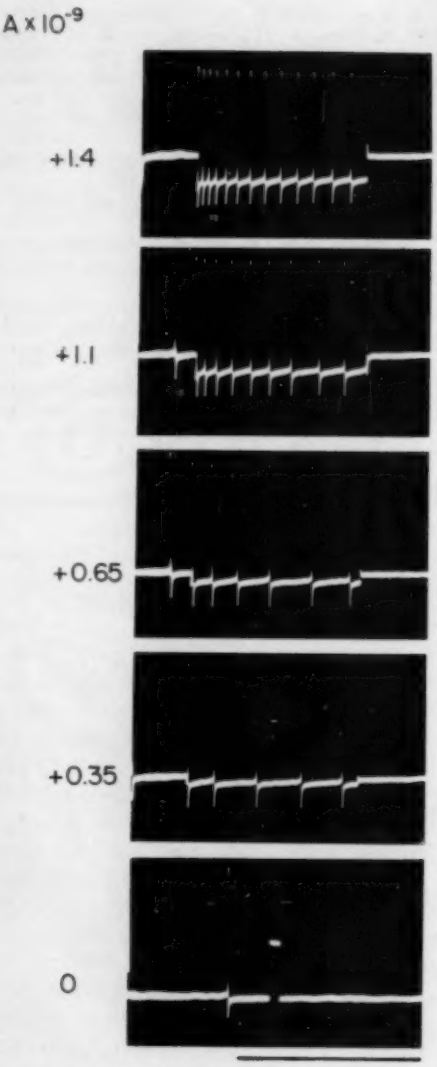

Fig. 5 (Lipetz). Response of the same cell of Figure 4 to depolarizing current pulses. Current is applied through same intracellular microelectrode used for recording. Figures at left give intensity of depolarizing (microelectrode +) currents. Time line: 1.0 sec. Square wave in bottom record is 20 mV calibration.

and this value differs considerably from the values of either 1.0 or 300 msec. found by Dr. Lipetz.

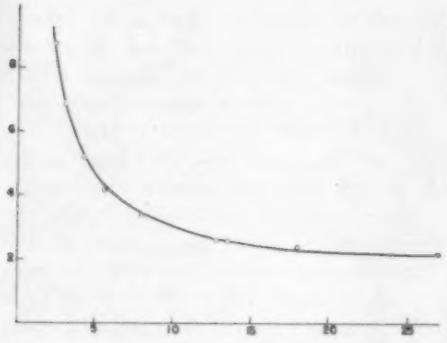

Fig. 6 (Lipetz). Strength-latency curve in another cell. Ordinate: current intensity in A × 10^{-9}. Abscissa: time required for firing of first impulse (msec.). Current was applied by means of intracellular microelectrode.

If we still have time, I should like to show a final slide (fig. 7) which illustrates some features of the interaction between light and currents through the microelectrode. In this experiment the current was applied 10 seconds before taking each picture and the values of current intensity are given at the left. In the left hand column the current was depolarizing (electrode positive) and in the right hand column it was hyperpolarizing. Light intensity was constant throughout. In brief, it is seen that when the cell is depolarized light evokes a smaller depolarizing potential but the frequency of firing is higher. Vice versa, if the cell is hyperpolarized the "relative depolarization" evoked by light is larger, but the frequency of firing is slower or firing may be altogether prevented. While it may be premature to attempt an interpretation of all of these findings at the present time, I venture to think that the observation that the size of the slow potential evoked by a light of fixed intensity

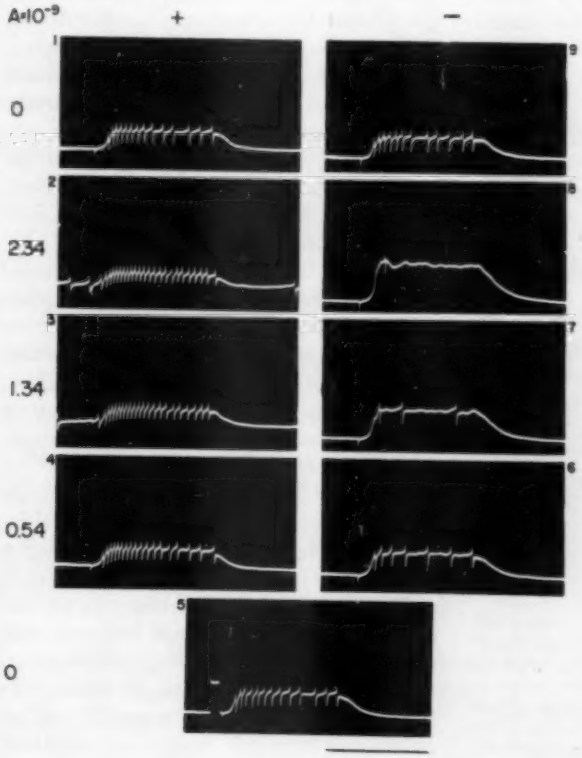

Fig. 7 (Lipetz). Interaction between light and currents through microelectrode. The eye was illuminated for one second in each record. Light intensity was constant throughout but polarization of the cell membrane was changed in the different records by means of currents through the impaling microelectrode. Numbers at left give current intensity in A × 10^{-9}. The records were taken in the order indicated by the small numbers 1 to 9. Records 2 to 4: depolarizing currents. Records 1, 5, and 9: controls. Time line: 1.0 sec. 20 mV calibration in Record 5.

depends upon the polarization of the cell membrane may be of some interest.

RUSHTON: I have never worked with Limulus but the strength-duration curve is an old friend. If one employs two stimuli with the strengths and durations shown by the points A and B in Figure 8 then A will excite the mechanism with short latency only and B will only excite the long-latency mechanism. By combining A and B together in various proportions it could be ascertained whether these two mechanisms are quite independent or whether connected through electrotonic spread, or otherwise, and if so, how closely. Also, if the latencies from shocks A and B were sufficiently constant, it might be possible by altering the interval between these two shocks to determine the time of nerve conduction between the two loci of their action.

LIPETZ: I would like to thank Dr. Rushton for the suggestion. It shows the benefits of bringing together people from somewhat different disciplines. I can answer this question only in part. From other observations I know that there is some interaction. If one stimulates the ommatidium with currents of very low intensities, one obtains only a "late response" consisting of a single spike. Its latency decreases with higher intensity until one second spike will appear and then a third and so on. The relation between current intensity and latency for each spike follows regular curves and there are sometimes characteristic ratios of the latency differences between different spikes. At some particular threshold the "early response" appears. In some cases, when this happens, the latency of the "late response" spikes increases. In a few preparations it was observed instead that the latency of the "late response" spikes decreased. On these grounds I should think that there is some interaction between the two types of electric response.

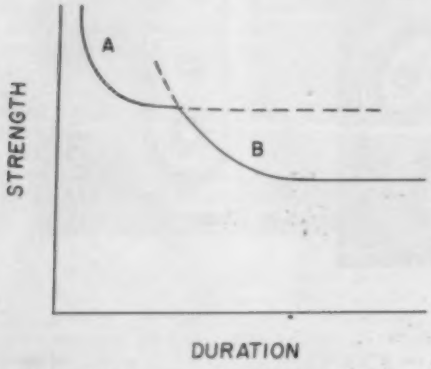

Fig. 8* (Lipetz. The strength-duration curve.

* Figure 8 was presented by Dr. Rushton.

KUFFLER: I should like to ask the chairman a question. Did you try antidromic stimulation while recording the potentials such as you showed in your last slide? I think that this experiment could show where impulses originate following direct current stimulation. If the size of antidromically evoked spikes were different from that of spikes evoked by direct current stimulation, then one would have a good presumptive evidence that current-evoked spikes do not originate near the stimulating electrode. Do you agree with that?

FUORTES: No, I do not agree but I did not use antidromic stimulation.

KUFFLER: But why do you disagree with my suggestion?

FUORTES: Perhaps I did not understand your suggestion, but if I saw that spikes evoked by electric stimulation differ from antidromic spikes I could perhaps conclude that the impulse originates in different places in the two cases, but not necessarily that the current-evoked spikes originate at a distance from the microelectrode.

WAGNER: If I remember correctly the experiments I performed with Dr. Hartline and Dr. Coulter, strong current strengths applied continuously were not as effective as light in setting up sustained trains of impulses. Something appeared to be shutting off repetitive firing initiated by strong currents. Dr. Lipetz's work points toward two separate mechanisms and suggests that impulses may originate in different locations.

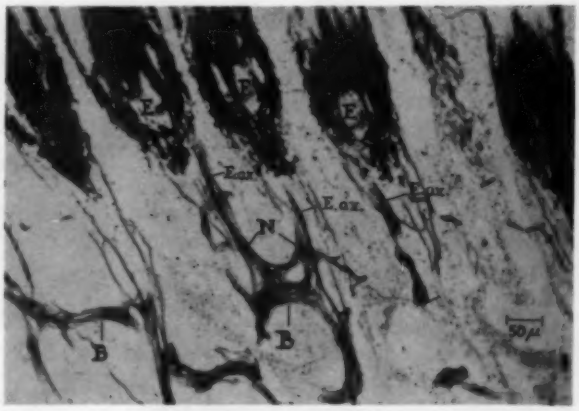

Fig. 9* (Lipetz). Light micrograph of compound eye of Limulus, 10 μ paraffin impregnated section, Samuel's silver stain. The heavily pigmented ommatidia are seen at the top of the figure. The unstained cell bodies of three eccentric cells (E) give rise to axons (E. ax.). Axons of retinula cells are also stained (dense markings parallel to eccentric cell axons, but thinner). Bundles of branches from retinula and eccentric cell axons (B) converge in regions of neuropile (N) near eccentric cell axons.

It occurred to me that one may, perhaps, be able to stir up the contents in one omatidium in such a way as to damage one of the locations, like the soma of the eccentric cell and not the axon. After such a procedure, it is possible that only the early response will remain.

RATLIFF: I should like to point out that with electric stimulation you may obtain quite different results than with light because you are stimulating the whole eye, not just the element from which you are recording.

The first slide (fig. 9) shows (as Dr. Lipetz mentioned) that there is an extensive plexus of lateral interconnections just behind the ommatidia. As one increases the stimulating current more and more of the ommatidia are stimulated, and the resulting inhibitory interaction which takes place by way of the plexus will greatly modify the response of each and every one of them. In the case of light stimulation this problem is easily avoided by confining the illumination to a single ommatidium. I think it is important to keep in mind the fact that the inhibitory interaction may significantly affect the results of your experiments, particularly at high intensities of electric stimulation.

Dr. Miller could not be here today and I am taking the liberty of showing some of his slides to illustrate a few more details of the structure of the Limulus eye.

The next slide (fig. 10) shows the microstructure of the rhabdomeres. I think it is incorrect to say that their structure closely resembles that of the vertebrate rods. While it is true that in some planes of section they

Fig. 10 (Lipetz). Electron micrograph of Limulus ommatidium in transverse section. Parts of 12 wedge-shaped retinula cells are seen symmetrically arranged about the dendritic distal process of the eccentric cell. The spokelike rays of the rhabdom are composed of the rhabdomeres of the retinula cells. (Enlargement, ×5,000.) (Set Miller: J. Biophys. & Biochem. Cytol., **3**:421–428, 1957).

* Figures 9 through 13 were presented by Dr. Ratliff.

have a laminar appearance like the vertebrate rods, in three dimensions they resemble a honeycomb and are quite unlike the three dimensional appearance of the vertebrate rods.

The next slide (fig. 11) shows this a little more clearly. If the section is cut transversely through the ommatidium one sees a striated structure which does, superficially, resemble the vertebrate rod. If the section is cut obliquely, however, it is evident that the structure is composed of tubes. If the section is cut parallel to the optical axis, one sees the tubes end on. These tubes are actually microvili extending out from each of the retinula cells, and one can see near the center of the top section, an irregular line, showing where the tubes join.

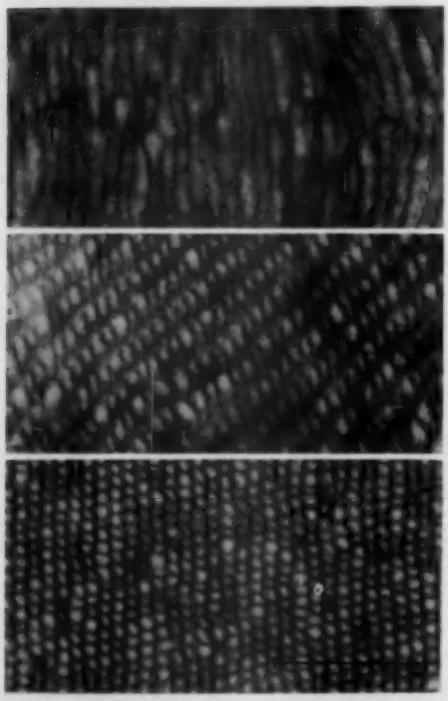

Fig. 11 (Lipetz). Electron micrographs of Limulus rhabdomere. Top: Appearance of the rhabomere in transverse section. Arrows indicate horizontal markings in zone where microvilli from neighboring retinula cells abut. Middle: Rhabdomere in oblique section. Bottom: Rhabdomere in which the microvilli have been cut squarely across. (From Ratliff, F., Miller, W. H., and Hartline, H. K.: Neural interaction in the eye and the integration of receptor activity. Ann. New York Acad. Sc., **74**: in press, 1958.)

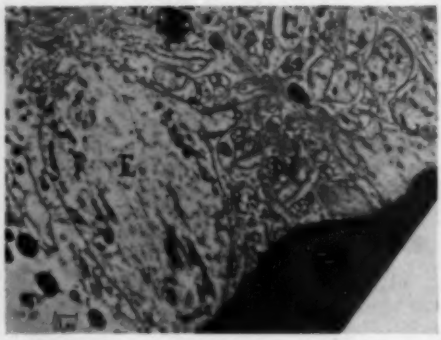

Fig. 12 (Lipetz). Electron micrograph of eccentric cell axonal branch. The eccentric cell axon (E) has been sectioned transversely and has an oval outline. A small branch (b) is in continuity with the axon. Near the axon is a region of neuropile (N) composed of axonal branches of eccentric cell and retinula cell axons. (From Ratliff, F., Miller, W. H., and Hartline, H. K.: Neural interaction in the eye and the integration of receptor activity. New York Acad. Sci., **74**: in press, 1958.)

The next slide (fig. 12) shows the fine

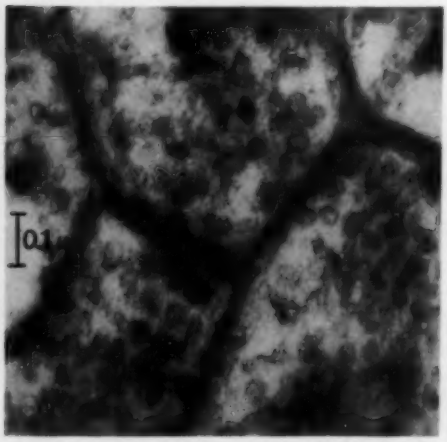

Fig. 13 (Lipetz). Electron micrograph of region of neuropile. Parts of five axonal branches can be seen. The dense-bordered closed outlines 200 to 500 Å in diameter are vesicular structures similar to those seen in synaptic areas of animals from many phyla.

branchings of the axons from the retinula and eccentric cells. These branches come together and form clumps of neuropile in which there appear to be synaptic connections.

The last slide (fig. 13) shows such a region. The dark bands are the junctions of the small branches. I think that these anatomic details, obtained by Dr. Miller, may be of help in interpreting the results of your experiments on electric current stimulation.

FUORTES: Thank you very much, Dr. Ratliff. The next paper on the program is by Dr. Donald Kennedy and its title is "Responses from the crayfish caudal photoreceptor."

RESPONSES FROM THE CRAYFISH CAUDAL PHOTORECEPTOR*

DONALD KENNEDY, PH.D.
Syracuse, New York

The impressive array of photoreceptor types found among animals is very tempting to the comparative physiologist, whose function it is to examine the differences—and similarities—between the ways in which organisms perform equivalent physiologic functions. Such studies on the visual system have already met with considerable success in elucidating basic problems of function, the work of Hartline and his co-workers on the Limulus ommatidium being the major case in point. This paper will describe some experiments on another arthropod photoreceptor, which appears to have quite a different function from that of other visual systems.

The sense organ used in these experiments is found in the sixth abdominal ganglion of the crayfish, which lies—like the rest of the ventral nerve cord—directly above the translucent exoskeleton of the ventral abdominal segments. Prosser (1934) first noted that illumination of this ganglion would cause sustained increases in the spontaneous activity characteristic of the intact nerve cord.

* From the Department of Zoology, Syracuse University. This investigation was supported by a grant (PHS B-1269) from the National Institute of Neurological Diseases and Blindness, Public Health Service, Bethesda, Maryland.

METHODS

Excised cords were stored in cold van Harreveld's solution for 12 to 30 hours before recording in order to eliminate spontaneous activity. In such preparations, the activity of photoreceptor afferents may be recorded with gross wick electrodes in the absence of spontaneous discharge. In addition, the activity of single fibers has been recorded in intact prepartions after isolation by fine dissection from the region between the sixth and fifth ganglia. The responses obtained by both methods are identical, except that the afferent fibers are spontaneously active at constant frequency in the intact preparations, but only occasionally so in excised cords. In the excised preparation, very few active fibers are present. As judged by the discharge pattern, there are usually only two, occasionally more. Though it cannot be said with certainty that the afferent units studied are primary ones, it seems probable that they are for the following reasons: (1) The discharge pattern is quite simple, and resembles that from some other first-order sensory neurons; (2) activity in these fibers is unaffected by treatment which abolishes the activity of nearly all interneurons in the cords; and (3) recording from other single units in the cord has demonstrated that in-

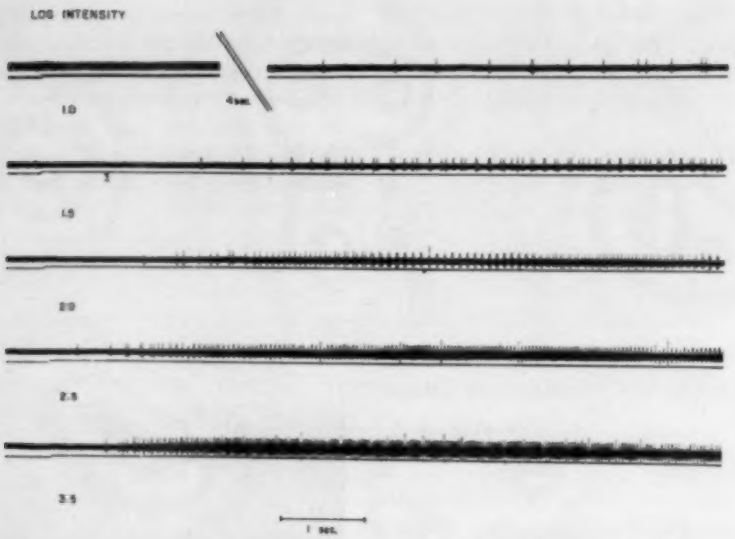

Fig. 1 (Kennedy). Response of an excised preparation in reduced-potassium solution to increasing intensities of continuous stimulation. Preparation showed occasional spontaneous activity; the first impulse in the fourth record is a spontaneous one. Bottom trace is the output of a monitoring photocell. Intensity of 1.0 = approximately 10 foot-candles in all figures. In this and succeeding records, the spikes have been slightly retouched for photographic purposes.

terneurons which are under afferent drive from the photoreceptor have a markedly different pattern of response.

In many experiments, reduction of potassium in the recording solution was also used to eliminate spontaneous activity. The sheath of the ventral nerve cord apparently is a barrier to the diffusion of this ion, and as a result the effects of a potassium-free solution are expressed gradually over a long period of time. In all cases in which low-potassium solutions were used, thresholds and discharge pattern for photoreceptor afferents were identical to those observed in normal van Harreveld's solution.

Several species of crayfish (including Procambarus clarki, Orconectes virilis, and Cambarus bartoni) were used, with no differences in the results.

Results

The discharge of impulses in afferent photoreceptor fibers is characterized by very long latency, slow adaptation, and pronounced after-discharge. Figure 1 shows responses from an excised cord to a series of prolonged exposures of increasing intensity. In this preparation, it appears that two fibers were active; this is suggested by the more or less regular "beats" produced by their discharges, which are of slightly different frequency. While frequency of firing shows an increase as the stimulus intensity is raised, latency decreases in more or less exponential fashion. Figure 2, taken from a similar series of records, illustrates that latency and frequency show quite different relationships with log intensity.

Below a critical duration of about 1.5 sec., discharge frequency of the response is constant if the product of stimulus intensity and duration is constant. In the duration range of 0.1 sec. to 1.5 sec., however, such reciprocal stimuli produce responses of very different latency (fig. 3). In such cases, latency to the shorter, more intense flash is always

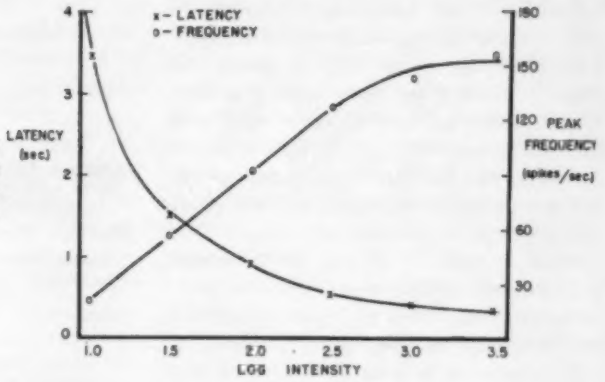

Fig. 2 (Kennedy). Latency and discharge frequency of response versus log stimulus intensity; data from an experiment similar to that in Figure 1.

shorter, even though both stimulus durations are shorter than either latent period. Such a discrepancy between critical durations for discharge frequency and for latency was mentioned by Hartline (1934) for the Limulus eye. It suggests that whatever mechanism determines latency operates over only a short time with respect to the duration of the latent period itself.

The records of Figure 3 illustrate another feature of the sensory discharge. At higher intensities, such as were used in this experiment, most preparations show an initial "fast" discharge, followed by a postexcitatory depression and then a secondary increase in frequency. The frequency of discharge in the initial burst, and the extent of the ensuing silent period, increase with intensity. Such discharge patterns are commonly seen in sense organs driven beyond their "normal" physiologic range, and are presumably akin to the phenomenon of cathodal block in nerve (Eyzaguirre and Kuffler, 1955).

Even at moderate intensities, after-discharge is a prominent feature of the response pattern of this receptor. A brief stimulus less than one $\log_{10}$ unit above threshold will routinely produce discharges lasting for 10 to 30 seconds; intense flashes can produce firing which persists for several minutes. During constant illumination, adaptation rates are quite low. Some preparations have sustained impulse discharge under these conditions for nearly an hour with a frequency drop of only about 20 percent. It appears possible to attribute this exclusively to light adaptation in the photochemical system rather than to the kind of neural adaptation found in other sense organs.

Considering the tonic discharge pattern characteristic of this photoreceptor, it is not

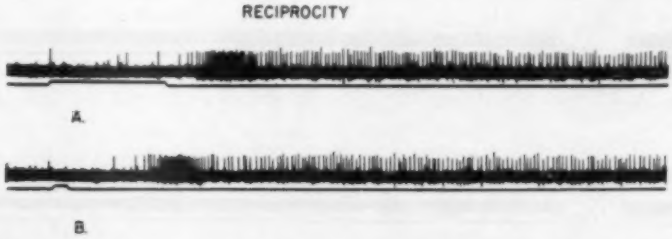

Fig. 3 (Kennedy). Excised preparation. Stimulus duration in A, 1.0 sec.; in B, 0.1 sec. Intensity of B 10 times A. Peak frequency and stable frequency in both responses were identical. Preparation showed occasional spontaneous activity.

surprising to find that subliminal flashes will yield summated responses over time intervals which are quite long compared with those reported from other sensory systems. Figure 4 demonstrates a case of such summation. When brief, paired flashes are adjusted so that they are barely below threshold in intensity, a summated response of one spike will occur in most preparations at an interval of 10 to 12 seconds. As the interval is decreased, the discharge in response to the second flash increases in number of impulses and frequency, and decreases in latency.

It is of interest to compare some of these constants from the caudal photoreceptor of the crayfish with similar values obtained for the Limulus ommatidium. Table 1 presents such a comparison. The Limulus data have been taken from the work of Hartline and his collaborators; in cases where the specific constants were not given, a range of values has been estimated from published figures.

It is evident from these data that the caudal photoreceptor of the crayfish exhibits a startling lack of the temporal resolving power found in the Limulus ommatidium. It is, unfortunately, too early to say with any certainty whether these differences should be attributed to a different sort of photochemical system, or whether they arise from neural characteristics. Some evidence favors the latter view. Measurements of the spectral sensitivity of the caudal photoreceptor agree quite well with those for the Limulus eye (Graham and Hartline, 1935); and both receptors show similar kinetics of dark adaptation. The neurons of arthropods, furthermore, vary widely in their tendencies to fire repetitively, as demonstrated in insect neuromuscular systems by Hoyle (1957) and in crustacean motoneurons by Adelman (1956). In view of such findings, it would be dangerous to conclude that the type of

TABLE 1

COMPARISON OF LIMULUS WITH CRAYFISH

	Limulus Ommatidium	Crayfish Caudal Photoreceptor
	(seconds)	(seconds)
Maximum latency (prolonged stimulus at threshold)	Below 1.0	8.0–12.0
Minimum latency	0.07	0.3
Critical duration for reciprocity (frequency used as criterion)	0.1	Between 1.0 and 2.0
Critical duration for first-spike latency*	0.05	Above 0.2
Maximum summation interval	1.0	12.0
After-discharge following prolonged stimulus†	0.1 sec. (2 impulses)	60.0 to 120.0

* Dependent upon intensity; the values given are examples (Hartline et al., 1952; Hartline, 1934).

† These values are difficult to compare, and depend upon the intensity used. In the Limulus ommatidium, a brief, very intense flash can elicit long after-discharge. Each figure given is for a case in which a prolonged stimulus had produced a stable frequency of 40 impulses/sec. at the time illumination was ceased (Hartline et al., 1952).

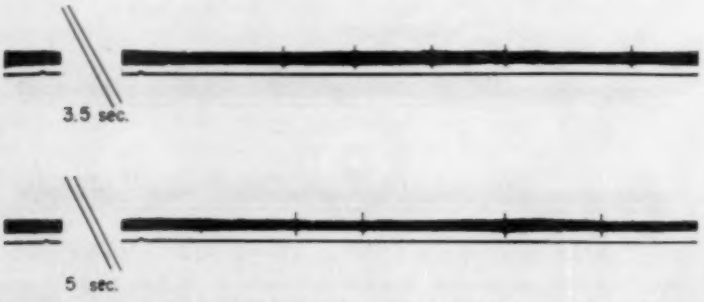

Fig. 4 (Kennedy). Summation of subliminal flashes. Stimulus duration 0.02 sec.; interval 3.5 sec. in upper record, 5.0 sec. in lower. The stimulus intensity (same in both records) was adjusted to a level where a single flash barely failed to elicit a single impulse.

discharge found in this receptor must be due to a unique visual pigment chemistry.

Data on the central pathways utilized by the caudal photoreceptor suggest that its function is to provide information on the absolute value of light intensity over long periods of time. In a series of experiments, single fibers between the sixth and fifth ganglia have been dissected, and their ascending discharge in response to various sorts of afferent drive studied. Illumination of the photorecptor produces tonic changes in the firing rate of smaller, spontaneously active central units. The larger fibers, including the giants, are not spontaneously active, and are never affected by photoreceptor discharge; the same is true of large motor neurons innervating appendages or abdominal flexor muscles. These central effects are in sharp contrast to those mediated by the compound eye, which when suddenly illuminated or stimulated by movement in the visual field elicits brief, phasic discharges in giant fibers and large motor fibers. Ascending activity in large abdominal interneurons is generally initiated by tactile stimuli, and is phasic in pattern. In some cases, spontaneously active interneurons have been found which are activated caudally both by photic and tactile stimuli; in rare cases, these modalities interact in such a way that illumination of the caudal photoreceptor inhibits both spontaneous activity and the phasic response to natural tactile stimuli.

This type of central activity is appropriate for a sensory system which functions as a long-term monitor of light intensity. The behavior of crayfish deprived of their compound eyes in light gradients is in agreement with these findings; Welsh (1934) has shown that such animals aggregate in the dark through a simple photokinetic response caused by increased locomotor activity when illuminated.

Discussion

The more recent comparative studies on the Limulus lateral eye (Hartline et al., 1952) and on the responses of vertebrate ganglion cells (Kuffler, 1953) have served to emphasize some similarities between these photoreceptor systems. In each, the single unit provides a sensory message with excellent temporal accuracy; and, in each, the response pattern of a unit may be affected by the activity of its neighbors. The study of other receptor systems, however—particularly mechanoreceptors—has revealed that there is often a differentiation of response type. "Fast" receptor units are found in which the discharge pattern emphasizes temporal accuracy and/or rate of change; in others, the "tonic" or "slowly-adapting" receptors, a relatively stable discharge frequency is found which gives information about the absolute value of stimulus intensity over rather long periods of time. Such differentiation of receptor types has been noted in crustacean muscle receptor organs (Eyzaguirre and Kuffler, 1955) and in the lobster statocyst (Cohen, 1955) among others.

The function of such complex photoreceptor systems as the vertebrate retina and the arthropod compound eye is to activate many channels of incoming information which can be analyzed centrally into a meaningful pattern. It is no surprise, therefore, to find that the individual units of such receptors really belong in the "fast" category—through the Limulus ommatidium may show rather slow adaptation. The properties of the caudal photoreceptor of the crayfish clearly place it in the "slow" class, and the relationships it maintains with central units are consistent with the type of steady-state information which it provides. It may be hoped that the "slow" characteristics of such a photoreceptor will also prove able to answer some basic questions about the visual process itself.

Summary

Afferent fibers from photoreceptor elements in the caudal abdominal ganglion of the crayfish show a tonic discharge pattern, characterized by long threshold latency, low adaptation rate, and pronounced after-dis-

charge. Summation of brief, subliminal flashes takes place at intervals of up to 12 seconds. The activity initiated by this afferent system centrally is restricted to smaller interneurons, and involves slow changes in discharge rate. It never activates the giant-fiber system. It is suggested that the function of the caudal photoreceptor is to provide a constant record of absolute stimulus intensity; its activity thus resembles that of slowly adapting mechanoreceptors more than that of other photoreceptor systems.

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Discussion

GRUNDFEST: It is not necessary to assume that the axons are themselves "slowly adapting." On studying the properties of the septate giant axons of earthworm and crayfish (Kao, C. Y., and Grundfest, H., Fed. Proc., **15**:104, 1956; J. Neurophysiol., **20**:553-573, 1957; and in preparation), we found that the nerve cords have very complicated interneuronal linkages. A brief (0.1 msec.) stimulus can initiate a long-lasting discharge (0.1 to 0.2 sec.) involving activity to and from the septate giant axons. In crayfish these axons may then respond repetitively at high frequency (up to 400/sec.), and these responses may be initiated at different regions of the axon.

KENNEDY: In response to Dr. Grundfest's comment: I know about this work and it is very nice. I really did not mean to imply that any neuron property was necessarily restricted to the axon. My intent was to separate such properties from those of the photo-chemical system.

BENNETT: Have you tried the effect of antidromic stimulation during or before and after a light pulse? Any interactions, such as resetting of the firing rhythm, should provide information as to whether these are really first order neurons.

KENNEDY: I have begun to try some of this, but there are no results.

FUORTES: If there are no more comments to Dr. Kennedy's paper, I wonder if somebody would care to continue the discussion of the first paper. Dr. Grundfest wanted to make a comment on the paper by Dr. Lipetz.

GRUNDFEST: I suggest that the site of electrical stimulation which Dr. Lipetz is studying may be restricted even more than Dr. Lipetz has already done. The data Dr.

Fuortes showed on the Limulus receptor potentials indicate that there is an electrically inexcitable component generated probably in the dendrite of the eccentric cell. MacNichol's data on Limulus (MacNichol, E. F., Jr., in: Molecular Structure and Functional Activity of Nerve Cells, Washington, D.C., Am. Inst. Biol. Sci., 1956) also indicate this structure to be electrically inexcitable. This part therefore probably is not involved in Dr. Lipetz's stimulation experiments.

FUORTES: Would Dr. Lipetz like to say something in reply to this?

LIPETZ: If I understood Dr. Grundfest correctly, he suggested that the slow potential arose from a site which was not stimulated by passage of a current through the eye. This was not my feeling—I like to think of the slow potential and the spikes as definitely arising from different sites. There is plenty of evidence for that, but the long chronaxie of the "late response" makes me think that it arises close to the site of impulse generation and not at the site of the slow potential. The interaction between light and the "late response" also showed a very long latency, and again this, I think, suggests that the late response arises close to the point at which the currents from light receptors depolarize the eccentric cell.* Dr. Ratliff has pointed out that there may be a confusing factor in these electric stimulation experiments because they were done on the whole eye and did not eliminate the inhibitory effect of stimulation of neighboring ommatidia. It might be that these inhibitory activities from the neighboring ommatidia slow down the response in the ommatidium whose active fiber was being recorded. That may be partly responsible for the difference in chronaxie between my records and those of Dr. Fuortes.

FUORTES: Yes, this is possible, since the results I presented were obtained by stimulation of a single cell. I should think, however, that the difference between the chronaxie found by Dr. Lipetz, and the time constant measured in my experiments, is too great to be accounted for by interaction.

BENNETT: According to Dr. Lipetz, there is a resistive barrier around the ommatidium which the axon of the eccentric cell penetrates. This barrier means that for corneal-cathode stimulation current enters the axon on the central side of the barrier hyperpolarizing the membrane and leaves it on the corneal side, there producing depolarization, the opposite occurring with corneal-anode stimulation. MacNichol has evidence that light-evoked impulses initiate near the axon hillock, and Hartline and co-workers have shown that corneal-cathode stimulation augments a light response, while corneal-anode stimulation diminishes it. These facts are consistent with inward and outward currents as proposed by Dr. Lipetz. The short latency response to electric stimulation should be initiated at different sites for the two directions of current flow, on the corneal side of the barrier for corneal-cathode stimuli, and on the central side for corneal-anode ones. Owing to electrotonic decrement, depolarization produced by light would be expected to differ at the two sites, to be smaller on the central than on the corneal side. Thus light should be less effective in facilitating a corneal-anode than a corneal-cathode short latency response. Has Dr. Lipetz any evidence on this point?

LIPETZ: Right here I would have to ask Dr. Hartline if he remembers the results of his and Dr. Sten Knudsen's work, because they did measure the interaction of these early responses with light. I do not have detailed studies of that interaction using the two current directions.

HARTLINE: Low threshold responses to direct current through the ommatidia are

* Note added in proof: It is apparent that I misunderstood Dr. Grundfest. I agree that it seems likely that the distal process of the eccentric cell is not directly affected by the electric stimulation. It probably functions as a passive conductor of local action currents from the depolarized structures (for example, the light receptors during light stimulation).

usually obtained with cornea negative although there are differences from preparation to preparation depending on the orientation of the ommatidium. Some differences are found between the interaction of the long latency with light, as compared to its interaction with the fast response. One of the most interesting points is that there is a more marked swing in the opposite direction toward relative refractoriness with the slow response than with the fast. I think that Dr. Rushton's suggestion that the two aspects of electric stimulation be tested is extremely interesting. I might add one other comment, although it is not pertinent to Dr. Bennett's remarks really. Recovery to prolonged stimulation by electric current occurs within a fraction of a second, whether tested by electric shock or by a flash of light. Recovery to an equivalent discharge elicited by illumination is rapid if tested with an electric shock, but is very slow if tested with a flash of light. So evidently, although these two response mechanisms are acting somewhere in a common locus, one is complicated by the interposed photochemical processes. The study of the electric phenomena in this eye, I think, is a very welcome addition to Limulus physiology because it does bring us into contact with the people who talk about electric excitation of the nervous structures elsewhere.

ELECTRIC RESPONSES FROM THE ISOLATED RETINAS OF FISHES*

EDWARD J. MACNICHOL,† JR., M.D.
Baltimore, Maryland

AND

GUNNAR SVAETICHIN,‡ M.D.
Los Angeles, California

INTRODUCTION

Although the electrical activity of the vertebrate retina has been studied for almost 100 years (Holmgren, 1865, and Dewar and McKendrick, 1873) there is still considerable controversy over the origin of the various components of the electrical response. This is because in recording with gross electrodes it is not possible to localize the source of potentials to single cell types or cell layers within the retina. It was only possible to obtain indirect evidence by comparing the responses of normal retinas with those of retinas damaged by diseases or poisons that selectively destroy specific cell types. The microdissection of single optic nerve fibers developed by Hartline and Graham (1932) and Hartline (1938) and the metallic microelectrodes used by Granit and Svaetichin (1939) permitted recording of the spike activity of single ganglion cells. However, these cells are third order neurons and although their responses reflect the activities of more distal structures they do not measure them directly.

The comparatively recent development of saline filled ultramicropipet electrodes (Graham and Gerard, 1946; Ling and Gerard, 1949; Nastuk and Hodgkin, 1950) has permitted the recording of slow electrical changes in highly localized regions of the retina, indeed it appear possible to measure the electrical changes taking place within single cells as has been done so successfully in

* From the Venezuelan Institute for Neurology and Brain Research (IVNIC).

† Present address: The Thomas C. Jenkins Department of Biophysics, The Johns Hopkins University.

‡ Present address: I.V.I.C., Apartado, 1827, Caracas, Venzuela.

other parts of the nervous system (Eccles, 1957; Frank and Fuortes, 1955), The earliest attempts at obtaining intracellular recordings from the vertebrate retina were not altogether successful. This was probably because the retinas were from frogs. In these animals the receptors, interneurons, and ganglion cells are very small. The micropipets do not record successfully from small cells presumably because they damage them too greatly.

Several years ago one of us (Svaetichin, 1953) found that fishes, which have much larger retinal cells than frogs, gave large electrical responses to light. These responses (10 to 30 mV) appeared to be intracellular since they were superimposed upon a negative resting potential (20 to 50 mV) which appeared and disappeared abruptly as the tip of the electrode was advanced a few micra. A curious feature of the responses was that they were negative in sign indicating a hyperpolarization of the cell membrane. This hyperpolarizing response to light has been confirmed in its essential features by several groups of workers: Mitarai and Yagasaki, 1955 (fish); Motokawa, Oikawa, and Tasaki, 1957 (cat and fish); Tomita, 1957 (fish); Grüsser,1957 (cat); and Brown and Wiesel, 1958 (cat).

The species of fish (Bream) used initially by Svaetichin (1953) when tested with flashes of light of different wavelengths gave negative responses throughout the visible spectrum. These responses were obtainable at two discrete depths in the retina separated by an interval of 20 to 30 micra.

In studies (Svaetichin, 1956) on another species of fish (Mugil) some of the retinal cells gave a similar hyperpolarizing response having a broad maximum in the yellow-green portion of the spectrum. In addition new and surprising types of responses to flashes of light of different wavelengths were frequently obtained. These responses were negative (hyperpolarizing) in the blue-green end of the spectrum and reversed in sign, becoming positive (depolarizing) in the yellow-red end of the spectrum. Similar responses have been described by Motokawa (1956), Motokawa, Oikawa, and Tasaki (1957), and Tomita (1958).

Elements having two such types of spectral response curves have been observed in the Mugil retina. One had a negative peak in the blue at about 450 mμ and a positive peak in the yellow around 600 mμ. The other had a negative peak in the green (490 mμ) and a positive peak in the red (650 mμ). The three types of response mentioned above were refered to as the "luminosity" (L), "yellow-blue" (Y-B), and "red-green" (R-G) types. These three types of response were clearly due to the cone system since they showed the very rapid light and dark adaptation typical of cone vision and could be elicited in portions of the retina from which the outer segments of the rods had been stripped away. They were at first believed to be the responses of the receptors themselves since they appeared to come from structures near the outer surface of the retina. It now appears that the responses originate not in the receptors themselves but in higher order neurons (MacNichol, Macpherson, and Svaetichin, 1957). This paper will describe recent studies of the intraretinal responses of several species of fish and localization of the electrode tip within the tissue by the use of histologic techniques.

Materials and Methods

Fishes of the families Centropomidae, Gerridae, Lutianidae, Mugilidae, and Serranidae were kept in oxygenated seawater until ready for use. After decapitation one eye was removed and the retina excised by cutting around the eye just posterior to the iris with sharp scissors, lifting the retina by the edges with watchmaker's tweezers, and cutting it free at the optic disc. The retina was placed receptor side up in a moist chamber having a transparent bottom and a sliding transparent cover which contained a small hole for the admission of the micropipet electrode. Oxygen that had been bub-

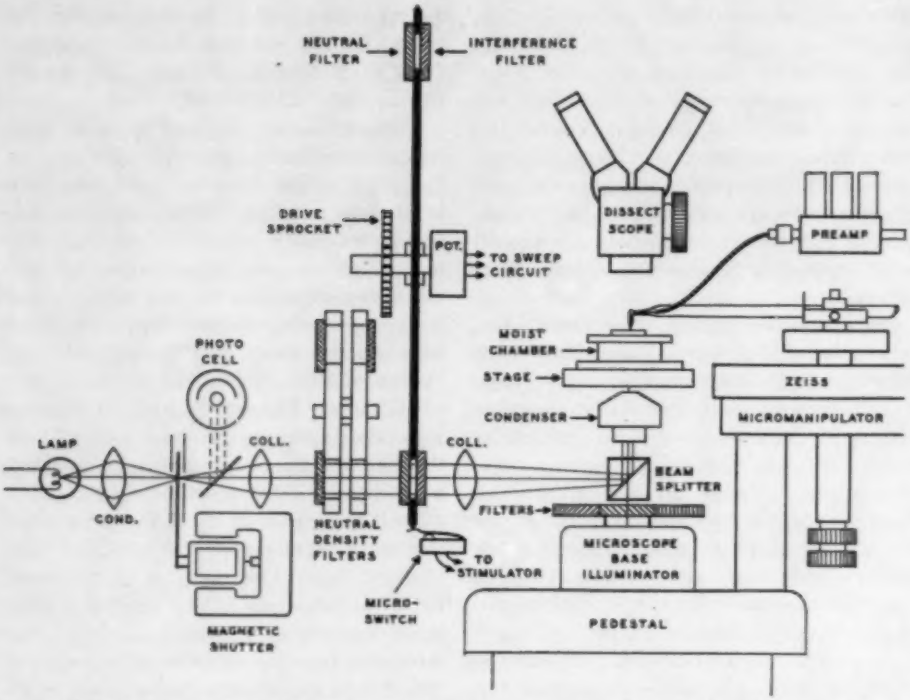

Fig. 1 (MacNichol and Svaetichin). Diagram of apparatus for stimulating and recording from isolated retina. Arrangement for supplying moist oxygen to retina not shown.

bled through slightly warm water was admitted continuously to the moist chamber. Electrical contact to the retina was made by means of a silver-silver chloride wire sealed into one end of a glass tube containing "frog-ringer" solution, which was closed at the other end by a cotton wick which touched the retina. In some experiments the pigment layer and outer segments of the rods were removed by touching the exposed retina with a strip of dry filter paper. No solutions were applied to the eye, which remained moist in the stream of wet oxygen.

It appeared to be essential for a successful experiment that no longer than five minutes should elapse between the removal of the fish from the aquarium and the insertion of the retina into an oxygen atmosphere. Good preparations were found to give electrical responses for at least one hour. Failure of the oxygen supply, which occurred on numerous occasions, caused rapid deterioration of the electrical responses. Temperature was not controlled in these experiments and was estimated to be in the range 25°C. to 30°C.

The apparatus was basically that described previously (Svaetichin and Jonasson, 1956) and is shown schematically in Figure 1. The moist chamber was mounted on the mechanical stage of a research microscope (Leitz "Laborlux") having a built-in illuminator. The optical system of the microscope was removed and substituted by a Zeiss Opton stereo microscope which was fitted to the vertical motion. The preparation was iluminated from underneath through the microscope condenser by two beams of light; one from the built-in light source and the other from a photostimulator. A beam splitting cube having a semireflecting diagonal sur-

face was used to combine the light from these two sources. A slide containing several interference filters could be inserted in the light path from the illuminator, which was used to furnish colored light for adaptation experiments in addition to illuminating the retina for examination.

The photostimulator operated as follows: a condenser lens concentrated the light from a tungsten filament lamp on the aperture of a magnetic shutter which was made by fastening a blackened balsa wood vane to an ink writer galvanometer (Sefram). A symmetrical aplanatic lens produced a collimated beam which passed through two wheels containing neutral density filters which attenuated the light over a range of six logarithmic units in steps of 0.3 L.U. This light then passed through the rim of a wheel containing a series of interference filters which permitted selection of 24 wavelengths covering the visual spectrum. Since filters could not be obtained at exactly equal wavelength intervals they were spaced around the wheel at distances corresponding to the difference in wavelength between successive filters. A potentiometer was driven by the wheel and furnished a voltage that changed linearly with wavelength which was used to furnish a wavelength sweep for an oscilloscope. The wheel could be turned continuously by an electric motor drive or it could be positioned manually.

Light passing through a filter was passed through another symetrical aplanatic lens which concentrated it upon the microscope condenser after reflection by the beam splitting cube. Although the condenser diaphragm permitted rough adjustment of the size of the spot projected upon the retina, accurate control of the area or position of the illuminated portion of the retina was not possible with this apparatus.

The microelectrodes were pulled from two-mm. O.D. pyrex tubing by means of a machine (Alexander and Nastuk, 1953) and filled with 2M KCl by first immersing their tips in distilled water until it rose by capillarity into the lumen of the electrode which was subsequently filled from the top with KCl. After 24 hours were allowed for diffusion, electrodes having a resistance of 10 to 50 megohms measured against frog ringer solution were selected. Electrodes having resistances of less than 10 meg. did not penetrate well and would not give persisting, large, localized potentials. Those having greater resistance than 50 meg. were usually found to be plugged. The electrodes were waxed to the drawn out tips of 8.0 mm. glass tubes that could be fastened in a Zeiss (Jena) micromanipulator. Chlorided silver wires connected to coaxial cables protruded into the micropipets. The cables were brought out through holes in the glass tubes and carried coaxial connectors that fastened to the preamplifier. Several of these electrode assemblies were prepared so that they could be quickly changed during an experiment.

A block diagram of the electric components of the stimulating and recording system is shown in Figure 2. The preamplifier was of the type described previously (MacNichol and Wagner, 1954). It contained circuits for voltage calibration and for measuring electrode resistance. The particular unit employed had a measured grid current of less than 10^{-12} amperes. The signal from the preamplifier was further amplified by two D.C. amplifiers (Offner). Both were connected to the vertical deflection circuits of a double gun oscilloscope (A.E.L.). One channel (low gain) was used to monitor the resting potential and was adjusted to give 100 mV full scale deflection. No sweep was used with this channel and the trace appears as an elongated spot at the right hand edge of some of the records. The other amplifier was adjusted so that the responses were of sufficient size to photograph and a zero offset was provided to bring the trace to the center of the oscilloscope screen. D.C. recording was used throughout the experiments. For some recordings an automatic rebalancing circuit designed by Macpherson was used

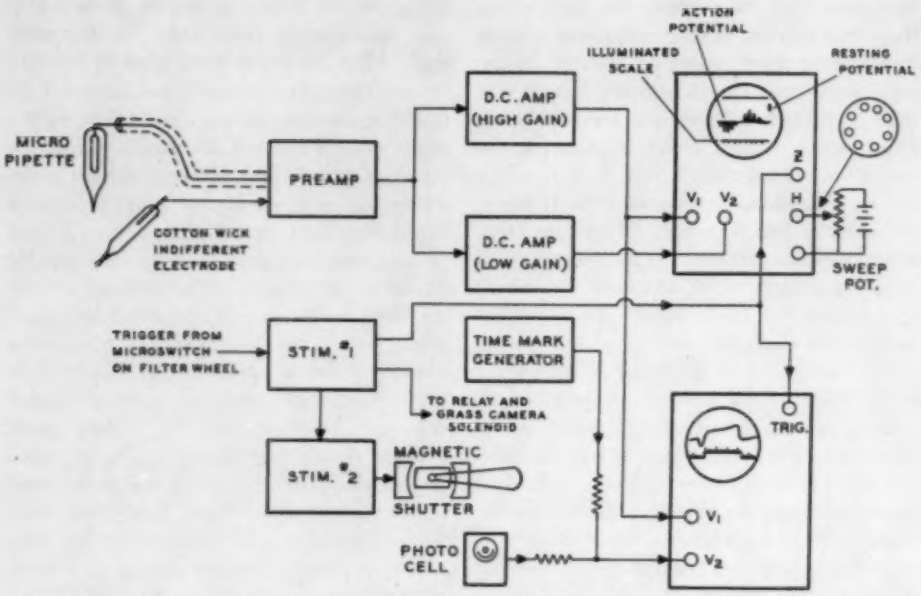

Fig. 2 (MacNichol and Svaetichin). Block diagram of electrical apparatus. Strobe light and counter circuits for identifying photographic records not shown.

which automatically set the trace to the center of the screen before stimulation. However, when a sufficiently small electrode was used and the retina was fresh and in good condition, the drift was so small that rebalancing was unnecessary during a series of measurements.

The high gain channel was also connected to an oscilloscope having an accurate linear trace (Tektronix 535), a dual trace input circuit was used to display both the response and a time trace from a crystal controlled time mark generator (Tektronix). Light reflected from a glass plate placed in the stimulating beam illuminated a photocell which provided a signal to deflect the time trace during illumination of the retina. Thus accurate measurements could be made of the latent period and times of rise and fall of the responses. An oscillograph camera (Grass) automatically photographed the screen of the oscilloscope used for waveform measurement. The double gun oscilloscope which presented the magnitude of the response as a function of wavelength was photographed by means of a manually operated 35-mm. camera (Contaflex). Two stimulators (Grass) were used for synchronization. One of these was triggered by a microswitch actuated when each interference filter came into position. It provided a time delay for the second stimulator, a trigger for the oscilloscope which had a time trace, and a brightening pulse for the other oscilloscope on which the spectral response curve was recorded. It also actuated the shutter of the oscillograph camera. The second stimulator actuated the shutter of the photostimulator. Numbering of the frames of film of each camera was accomplished by a pair of electric message registers which were illuminated by strobe lights.

Thus it was possible to obtain simultaneous records of the amplitudes of the responses as a function of wavelength (spectral response curves) and of the time courses of the individual responses.

Localization of the electrode tip in the ret-

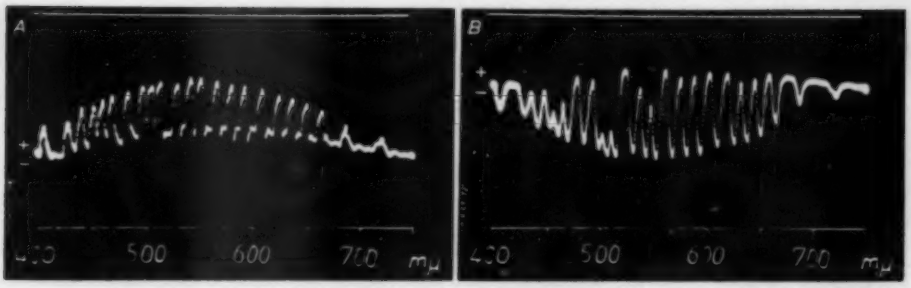

Fig. 3 (MacNichol and Svaetichin). Typical "luminosity" responses from fish retina (Gerridae). Record (A) obtained first after inserting electrode from receptor side; no resting potential, amplitude of response small. Record (B) obtained after further advance of electrode; resting potential about 20 mV; amplifier gain 1/10 that used to record A. Amplifier rebalanced between records so that resting potential does not show.

ina was done by filling the micropipets with a saturated solution of crystal violet in five-percent HCl (MacNichol, et al., 1957). After a typical recording from a given location in the retina a current of 10^{-7} to 10^{-9} amperes was passed for about one minute between the pipet and the reference electrode. This caused the dye to be forced electrophoretically from the electrode into the tissue and an expanding sphere of dye could be seen in the dissecting microscope. The size of this sphere could be controlled by adjusting the current and the time. Alcoholic solutions were found to remove the dye so that it was not possible to imbed the tissue in paraffin or methacrylate. Instead the tissue was fixed in 10 percent ammonium molybdate five-percent glacial acetic acid for 30 minutes, infiltrated with 20-percent gelatin five-percent glycerol just above the melting point of the mixture, and sectioned in the frozen state (Fernandez-Moran, 1952). Sections down to 25 μ in thickness showing good preservation of the retinal structures could be cut by this method. It is possible that other dyes or metallic pigments that are insoluble in alcohol could be used to permit the cutting of paraffin or methacrylate sections.*

* Tomita (personal communication) has recently been successful in marking the electrode position by a similar method. He filled his electrodes with potassium ferricyanide which was forced into the tissue electrophoretically. A color was developed by immersing the retina in ferrous chloride.

Experimental results

When the electrode was first placed in contact with the receptor side of the retina an electroretinogram would appear on the oscilloscope. This response had an amplitude of several hundred microvolts. A few retinas gave normal intraretinal potentials but no surface electroretinogram. When the electrode was advanced about 700 μ and the manipulator tapped with a finger to facilitate penetration of the electrode tip, the response shown in Figure 3-B often appeared. This presumably intracellular response started from a resting potential of 10 to 30 mV and was negative in sign often having amplitude of 20-30 mV. Occasionally a smaller, positive response without a resting potential as shown in Figure 3-A was obtained. This presumably extracellular response changed abruptly to the large negative type when the electrode was advanced slightly and the resting potential appeared. The spectral response curves of this type were characterized by a gradual change in amplitude with wavelength, having a maximum in the yellow-green region of the spectrum. These responses are referred to as L-responses.

In a number of experiments on the Mugil retina responses of the type seen in Figure 4-A were obtained. This complex spectral response curve is bulit up of diphasic responses which show large "off" effects. Ap-

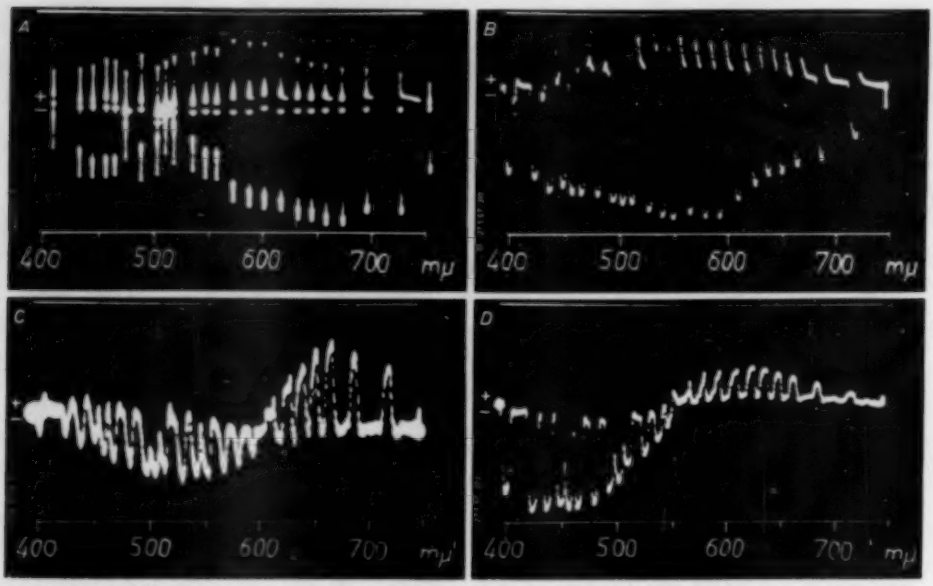

Fig. 4 (MacNichol and Svaetichin). Typical "luminosity" and "chromaticity" responses from fish retina (Mugil). This species of fish was the only one that showed both R-G and Y-B responses. Records (A and B) Luminosity responses. (C) R-G response. (D) B-Y response. Record A taken from retina from which pigment layer was not removtd; B, C, and D taken from another retina from which pigment was removed. All responses $>$ 10 mV but no resting potential in A which was presumably extracellular. B, C, and D had resting potential $>$ 20 mV (negative) and were presumably intracellular.

parently this curve represents a recording from a population of units having different spectral sensitivities. The submaxima are situated at about 450, 490, 570, and 650 mμ; that is, in regions of the spectrum which well correspond to the maxima of the spectral response curves of the L, R-G, and Y-B type, obtained by intracellular recording and presented in Figure 4-B, C, and D, which were all obtained from the same retina.

The L response (fig. 4-B) was recorded after introducing the electrode a few micra deeper than it was when recording a spectral response curve of the type seen in Figure 4-A. When the electrode was advanced 20 to 30 μ further into the retina a resting potential appeared, and a spectral response curve of the R-G or the Y-B type was obtained at this depth. After an additional advance of 6.0 to 8.0 μ there was an abrupt increase in the resting potential to −80 to −100 mV and all responses to light ceased. On several occasions this sequence of events was repeated in reverse order as the electrode was withdrawn, and again in the original order as it was reinserted. From the difference in the electrode depth between the appearance of the L response and the R-G and Y-B responses it was concluded that the cells from which the latter types of responses were recorded were located 20 to 30 μ more proximal than those that gave the L response. Furthermore, these responses were lost upon very slight movement of the electrode, whereas considerable motion could be tolerated before the L response was lost. Therefore it was concluded that the L responses arose from much larger cells than those responsible for the R-G and Y-B responses. In several experiments, the electrode was inserted from the anterior side of the retina and R-G or Y-B responses were obtained before the L response, as the electrode penetrated.

Of the various species examined only members of five different families were positively identified. Of these only the Mugil (Mullet) gave both the R-G and Y-B responses as shown in the bottom row of records in Figure 5. The Lutianidae (top record of Figure 5) and other unidentified species collected in water 30 to 70 meters deep gave only an L response that had a peak toward the blue end of the spectrum and a very small amplitude in the red. Of the shallow water fishes other than the Mugil the Serranidae gave both L and Y-B responses (Row 2 of Figure 5). The Centropomidae, on the other hand, gave L and R-G responses (Row 3 of Figure 5). Thus, it is evident that the types of responses were very dependent upon the species of fish used.

Figure 6 summarizes the data from a large number of experiments. The histograms show the number of responses observed at a given wavelength plotted as a function of wavelength for fishes belonging to four different families. The total number of responses with peaks in a given wavelength region is given by the numbers.

Two different types of experiments were done to obtain further inforamtion on the nature of the R-G and Y-B responses. The first of these showed the change in the time courses of the responses as a function of wavelength. At each wavelength a flash of light 0.3 seconds in duration was presented to the retina and the response recorded by both cameras. Figure 7 is a record of a typical experiment. The top record shows

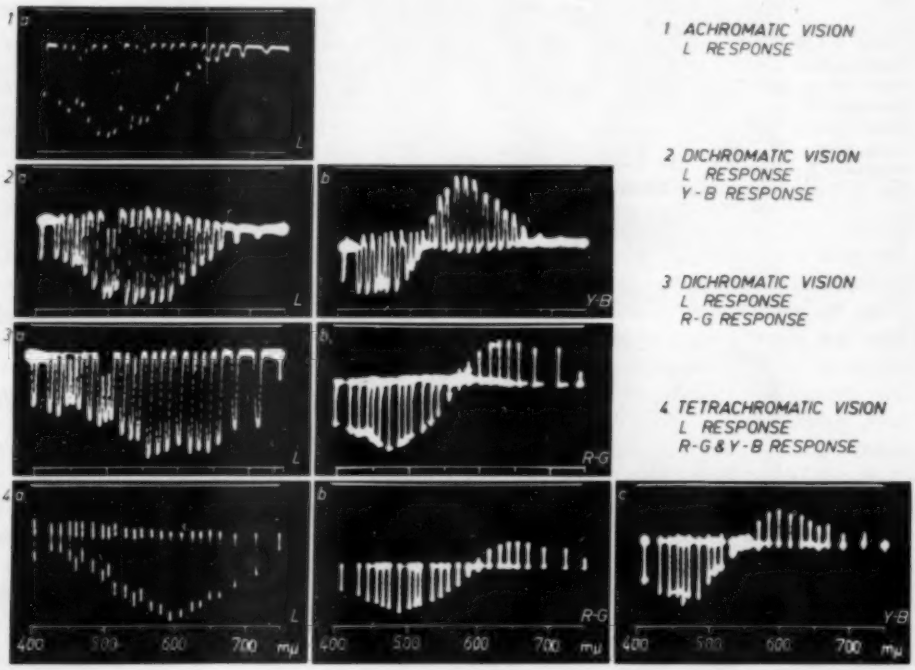

Fig. 5 (MacNichol and Svaetichin). Responses from retinas of fishes belonging to different families. (1) Lutianidae, which live deeper than 30 m., gave only L responses with peak at about 490 mμ. (2) Serranidae gave both L and Y-B responses. (3) Centropomidae gave both L and R-G responses. (4) Mugilidae gave all three types of response. The fishes used to make records 2, 3, and 4 were all caught in very shallow water. Gerridae, also shallow water fish, gave both L and R-G responses similar to (3) which are shown in Figure 8.

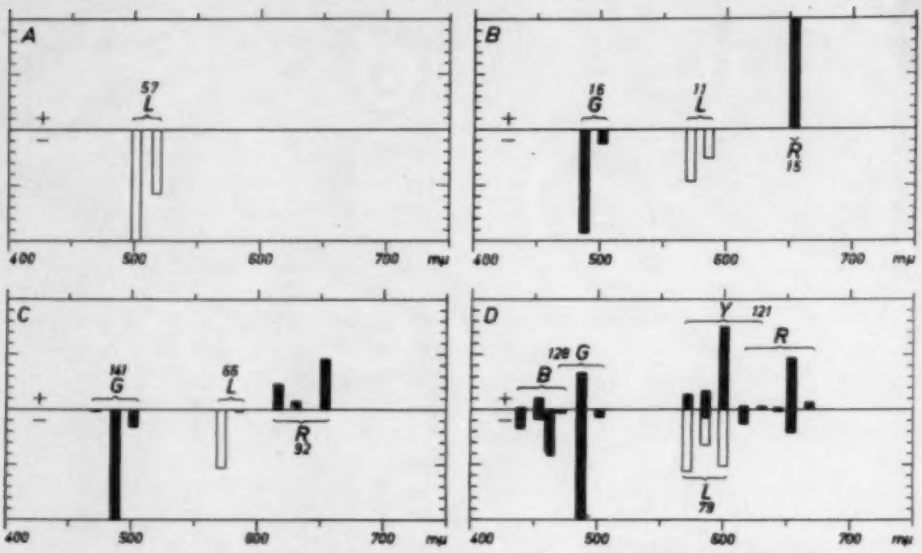

Fig. 6 (MacNichol and Svaetichin). Distribution of response maxima of all elements from which recordings were made: (A) Lutianidae, (B) Centropomidae, (C) Gerridae, (D) Mugilidae. The Lutianidae were caught at depths of 30 to 70 m.; all others in very shallow water. The figures above each bar line represent the total number of responses recorded having maxima in the indicated wavelength interval. The letters indicate the type of response, L = luminosity, G = green component of red-green pair, B = blue component of B-Y response, and so forth. The direction of each bar line indicates the polarity of the response. As shown in (D), a few responses were obtained in which reversed polarity was observed; that is, B and G responses positive and R and Y responses negative.

the peak amplitudes of the responses as a function of wavelength. The lower records show the individual responses in detail. The numbers identify corresponding traces on the upper records; negative numbers indicating shorter wavelengths and positive numbers indicating longer wavelength than the neutral point. The response is from a typical Y-B unit of the Mugil. It is evident that records taken with wavelengths much shorter than the neutal point have short latencies and a fairly rapid rise and fall and small overshoots. Those taken at wavelengths much longer than the neutral point have longer latencies, a slower rise and fall, and exhibit no overshoot. Those records taken near the neutral point show mostly overshoot. These records give further confirmation to the belief stated earlier (Svaetichin, 1956) that the R-G and Y-B responses appear to result from the subtraction of two opposed processes: A negative blue or green process having a short latency and a rapid rise and fall and a positive red or yellow process having a longer latency and a slower rise and fall. The total response in each record appears to be obtainable by taking the algebraic sum of two components each of constant shape but varying in amplitude.*

If this interpretation is correct it should be possible to find a means of inhibiting one of the components leaving the other unchanged. This has proved to be the case. By using an "adapting" light of a wavelength to which one of the processes is much more

* Some records have been obtained in which the R response is more rapid than the G type so that near the neutral point the initial "on" component is positive and the "off" component is negative (Svaetichin, 1956, fig. 1, p. 22 and fig. 4, p. 28).

sensitive than the other it is possible to suppress the corresponding component of the response without materially affecting the other component.

Figure 8 shows the results of such an experiment. The record (A) shows a typical R-G response (Gerridae). The spot at the right hand edge of each record shows the resting potential measured with the low gain channel. No adjustment of the amplifier balance was made during the experiment so that the position of the high gain trace also indicates changes in resting potential. The retina was then illuminated with light of 447 mμ and record (B) taken during this illumination. The effect of adapting light was to make the base line more negative, decrease the response at wavelengths shorter

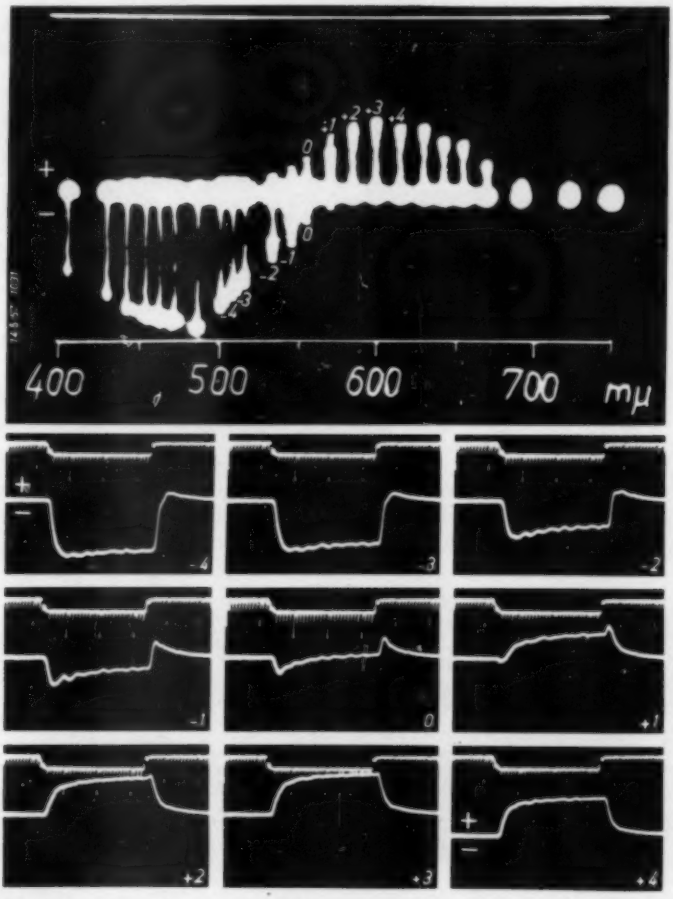

Fig. 7 (MacNichol and Svaetichin). Changes in time course of responses from a Y-B receptor of a Mugil retina as a function of wavelength. (Top record) Recording of response amplitude as a function of wavelength. (Lower records) Responses recorded as a function of time. Each numbered record was taken simultaneously with the response peak bearing the same number in the top record. Top trace in each record indicates time in tenths and hundredths of a second. Deflection of top trace is due to output of photocell circuit used to monitor light flash (duration 0.3 sec.). Records taken at approximately three-second intervals.

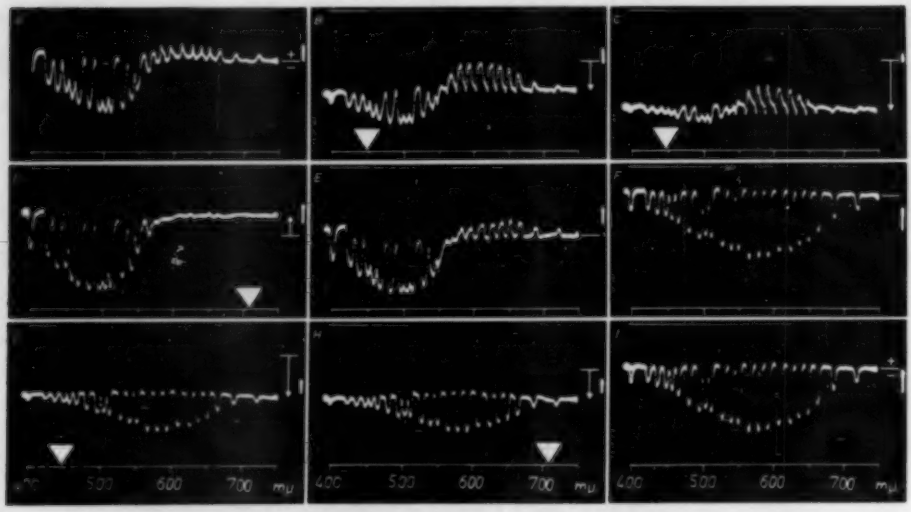

Fig. 8 (MacNichol and Svaetichin). Modification of responses from R-G and L units of Gerridae by colored adapting light. Modification of responses from R-G (ABCDE) and L (FGHI) units of Gerridae by colored "adapting" lights. Records A, E, F, I controls at beginning and end of each experiment. Records B, C, and G taken during adaptation with blue light of 447 mμ. The adapting light used in C and G was much stronger than in B. Records D and H taken with red adapting light of 710 mμ. The line at the right hand edge of each trace was made by the low gain channel and indicates the resting potential and the total amplitude of the response (distance between upper and lower horizontal lines 100 mV). The original resting potential is indicated in each record by the horizontal line segment drawn near the right side of each record. The arrow indicates the displacement in resting potential due to the adapting light. White triangles indicate adapting light used.

than the neutral point, and increase it at longer wavelengths. Stronger blue light nearly abolished the G component with little effect on the R component as shown in record (C). Red light of 710 mμ caused the baseline to go positive of the resting potential and abolished the R response as shown in record (D). Record (E) is a control showing that after the adapting light was turned off the responses and the resting potential were essentially the same as before it was turned on. Records (F), (G), and (I) show that the shape of the luminosity response from the same species of fish was not affected by colored adapting light; only the resting potential and the response amplitude being affected. Thus the R-G response was clearly made up of two separable components, whereas the L response appeared to consist of only a single one.

Since the R-G and B-Y responses appeared to originate at a depth in the retina nearer the front of the eye than the site of origin of the luminosity response it seemed unlikely that both were picked up from the receptor cells themselves as was originally assumed. It therefore became urgent to find out in which structures the responses originated. This was done by filling the micropipet electrodes with dye and marking many spots that gave the same type of response in small pieces of retina. These were subsequently sectioned and examined by transmitted light without additional staining.

Figure 9 shows the location of some of the dye spots which appeared bright purple in the original slides. The upper row of photographs show the locations of five L type responses. The dye spots appear to be in the outer plexiform layer just below the cone synapses. The lower row of photographs show the locations of the R-G and

Y-B responses which were clearly recorded from locations proximal to the L responses. The dye spots appear to be in the inner nuclear layer where the bodies of the bipolar cells are located. Time did not permit further more accurate localization using smaller spots of dye or a complete histologic study of the retinas of the fishes that were used.

Discussion

From the experiments that have been described it appears that what at first seemed to have been action potentials recorded directly from the cones were in fact recorded from second order neurons as previously suspected by other authors (Tomita, 1957). However, there is little doubt that the responses were produced by the cone system since the latent period was always very short; light and dark adaptation were very rapid; and the spectral sensitivity curves did not correspond to rods. Furthermore the responses could be

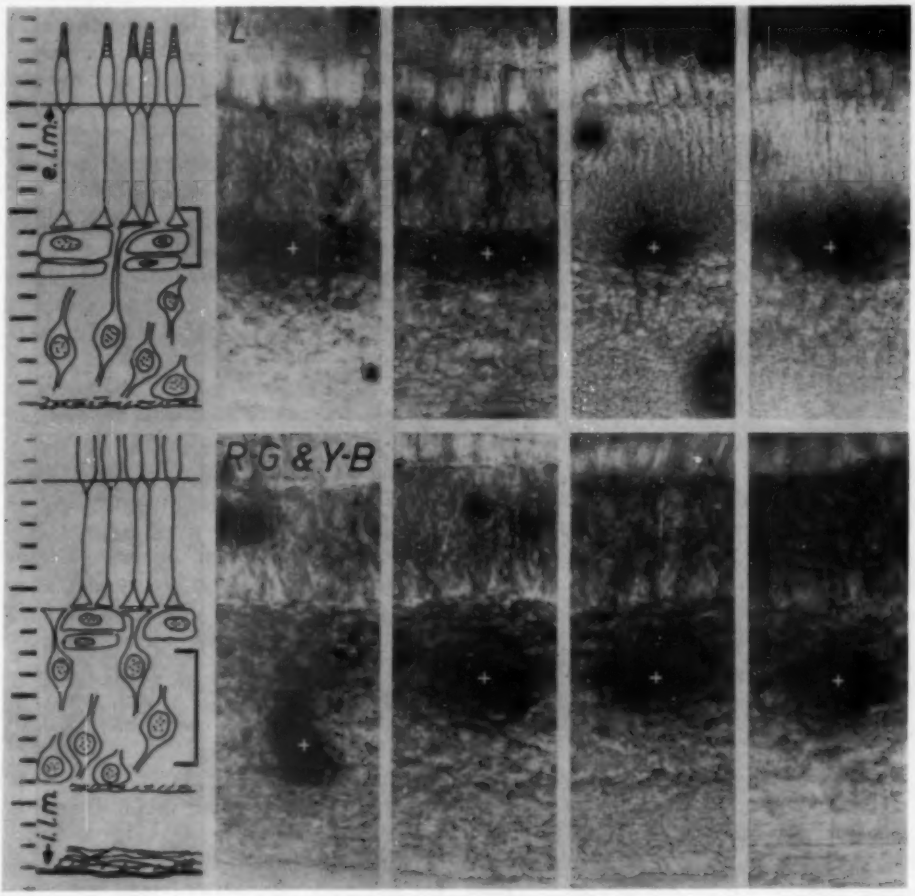

Fig. 9 (MacNichol and Svaetichin). Histologic sections of retinas of Serranidae and Mugilidae marked with dye driven electrophoretically from recording micropipet. Upper photographs = Location of origin of L responses. Lower photographs = Location of origin of R-G and Y-B responses. Drawings on left show structures schematically. e.l.m. and i.l.m. = external and internal limiting membranes. Distance marks = 10 μ.

found just as often in retinas from which the outer segments of the rods had been stripped off.

Although the amount the electrode was advanced after touching the retina was found not to be an accurate measure of its depth in the retina it appeared to give a good measure of relative depth once it had penetrated. Support for this statement is found in the very good agreement between the differences in depth of the dye spots corresponding to the L and R-G responses and the amount the electrode had to be adcanced between the appearances of these two types of response. This interval was found to be about 20 to 30 μ in both cases. The L response was maintained over a depth of at least 20 μ indicating that the electrode was in a large cell. Giant horizontal cells have been described in some fishes. Large cells can also be seen at the appropriate depth in our slides. Furthermore, Tomita, et al. (1958) have shown that there is an enormous amount of convergence in the L response of Cyprinus Auratus. He focussed spots of light on the retina and showed that the response continued to increase when the diameter of the spot was increased to many receptor diameters. He also showed that the shadow of a wire passing across the illuminated field had negligible effect and that the shadow of the edge of an illuminated field passing over the region of the micropipet tip caused a very gradual decrease in response. Thus it appears that the L response signals change in brightness diffusely over a large area of the retina. Unfortunately we have as yet no measure of the receptive fields of the R-G and Y-B responses. These remain to be measured. From the very small amount of electrode movement necessary to lose these responses they must arise from small cells. It seems strange that brightness should be signaled diffusely and color with greater acuity since brightness acuity is far more important in pattern vision. Just how these responses are related to the optic nerve message awaits a suitable technique for recording spikes from single ganglion cells or optic nerve fibers, which are few and small in these fishes. Grundfest (personal communication) has suggested experiments with chemical agents known to selectively block excitatory and inhibitor synapses. These experiments still remain to be done.

Another fact to be explained is the lack of spike discharges from the cells in which the responses were recorded. Either our treatment of the retina destroyed the spike producing mechanism or the bipolar cells pass their messages on to the ganglion cells by a slow, gradual depolarization, or by some unknown means. By recording from the ganglion cells or their axons it should be possible to find out whether or not all layers of the retina are functional in our preparation. The responses should also be re-examined in the living fish by a technique similar to that to be described by Brown and Wiesel later in this symposium. Perhaps impulses would be picked up from the inner nuclear layer if the retina were kept under more physiologic conditions.

Finally, it appears that we are no nearer to understanding the process of excitation in the receptors themselves. Either the receptors are electrically silent and the energy absorbed by the visual pigments mediates its effect in some way that does not involve a large separation or recombination of electric charges; or we have not yet been able to place our electrodes in a favorable location to record electric changes in the receptors.

Summary

The electrical responses picked up by microelectrodes inserted into isolated retinas of fishes and previously attributed to the receptors themselves have been shown to arise in more proximal structures. The response having a maximum in the middle of the visible spectrum (L response) was shown to originate in the outer plexiform layer and presumably arises in giant horizontal cells. The responses that show a negative polarity in the blue end of the spectrum and a posi-

tive polarity in the red end (R-G and Y-B responses) were shown to originate still more proximally in the inner nuclear layer. They were tentatively attributed to the bipolar cells. It was possible to fractionate the R-G and B-Y responses into two components by selective adaptation with monochormatic light. The L response, on the other hand, did not fractionate.

Only the L responses were picked up from some species of fish (all those caught at depths greater than 30 m.). All species showed L responses though the wavelength of maximum response varied with the species. Other species lacked the R-G response; others the Y-B. Only one species showed all three responses. As a tentative hypothesis it is suggested that the L response is a postsynaptic potential from a cell that has only excitatory synaptic endings whereas the R-G and Y-B responses are composed of postsynaptic excitatory and inhibitory potentials arising from excitation of pairs of different types of cones which have different spectral sensitivities.

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The unusually great efforts of Mr. Rojas who kept us supplied with live marine fishes, though we were a long way from the ocean, is also gratefully acknowledged.

We also wish to express our thanks to Dr. Fernández Yépez, ichthyologist, for identifying the species of fish that were used.

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Discussion

FUORTES: Thank you very much, Dr. MacNichol. I think it was well worth while to run a few minutes overtime. It is perhaps unfortunate that we have to cut down on the discussion time unless Dr. Wagner agrees to postpone his presentation by 10 or 15 minutes.

WAGNER: It is all right with me.

FUORTES: If necessary, then, Captain Wagner agrees to postpone his presentation by 10 minutes or so. I see many hands raised and I take note of this, but Dr. Forbes has registered for a special comment and he would like to present a few slides; then we'll proceed to the rest of the discussion, Dr. Forbes.

FORBES: First of all I would like to ask Dr. MacNichol if, in any of his experiments, he reversed the order of the observations starting at the red end of the spectrum to be sure that an element of light adaptation did not affect the results.

MacNICHOL: The direction of rotation of the filter wheel did not change the records of amplitude versus wavelength. Tomita has recently obtained similar records and, also in his case, no difference was noticed starting at one or the other end of the spectrum. One thing that I might mention is that in these particular mechanisms light and dark adaptations take place extremely rapidly. It's more like what has been called alpha adaptation in the human visual system. It is substantially completed in 40 or 50 msec., being very different from the slow adaptation one finds in the ordinary electroretinogram, unless the preparation is very old.

FORBES: My slides may not help to clarify these issues, but information may be worth presenting even if it poses new problems rather than solving old ones. We have recorded many ERGs of freshwater turtles and have recently made similar records from three species of lizard: horned toad, collared lizard, and sceloporus (tree lizard). In all we have recorded the gross ERGs, with one lead at the base of the eye and the "recording" electrode applied to some point in the retina. Figure 10-a shows the typical ERG of the turtle, regularly found immediately after excision in every turtle tested in the autumn and in all but a few apparently sick turtles in the spring; these few showed only the negative response (a-wave). In the horned toad, Chaffee and Sutcliffe (Am. J. Physiol., **95**:250, 1930) showed ERGs like that of the turtle, when they led off from the upper and lower segments of the retina, but negative potentials recorded from a band across the middle of the retina. We have not been able to place our recording electrode with the same precision as in their experiments, but Figure 10-b-e confirm their main finding of the marked difference due to location of the lead. Records b and c, from the same retina, show responses led from two points near the margin; d and e, from another animal, show the change due to recording from near the margin of the retina (e) and from near the fovea (d). Sections of the horned toad retina showed no rods, and further that the cones near the fovea are much smaller than those near the periphery.

Both with the collared lizard and the sceloporus, the first records, taken less than half an hour after decapitation, usually show

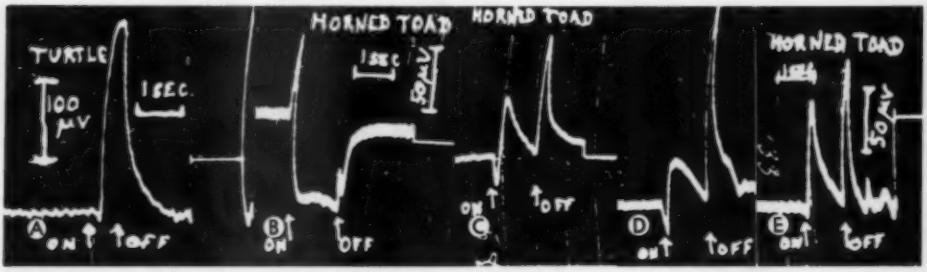

Fig. 10* (MacNichol and Svaetichin). (A) Typical ERG of turtle. (B-E) Horned toads, showing change in ERG due to change in location of recording electrode. (B and C) One retina. (D and E) One retina from another animal. (D) Lead near fovea. (E) Lead near margin of retina.

a large initial negative deflection followed by a very small positive b-wave. This b-wave then rapidly increases in successive tests until the on-effect resembles that in the turtle. This then persists for two or three hours and then decreases as the retina dies. This growth of the b-wave is shown in Figure 2. In contrast with the turtle, all three species of lizard show very large positive off-effects, see Figures 10 and 11.

Spectral sensitivity curves in all four species of reptile have been made from records with a monochromator. These are plotted as reciprocals of the relative quanta in flashes giving equal b-waves of a value approximately half the size of the maximal response. Figure 12 shows tentatively the comparison of the turtle and the three species of lizard. The turtle curve is from Deane et al.

* Figures 10 through 12 were presented by Dr. Forbes.

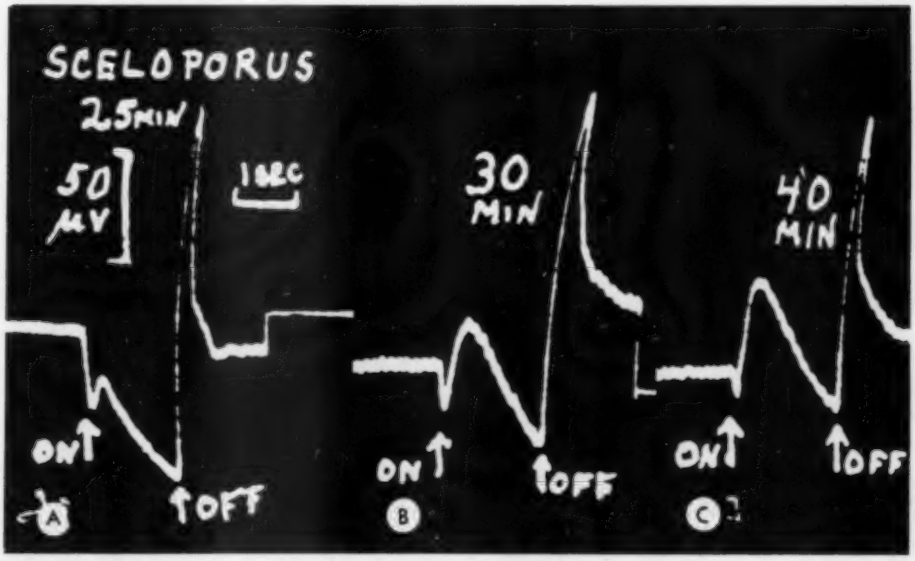

Fig. 11 (MacNichol and Svaetichin). Spiny tree lizard. ERG at three different stages of change after operation. (A) 25 minutes. (B) 30 minutes. (C) 40 minutes after decapitation.

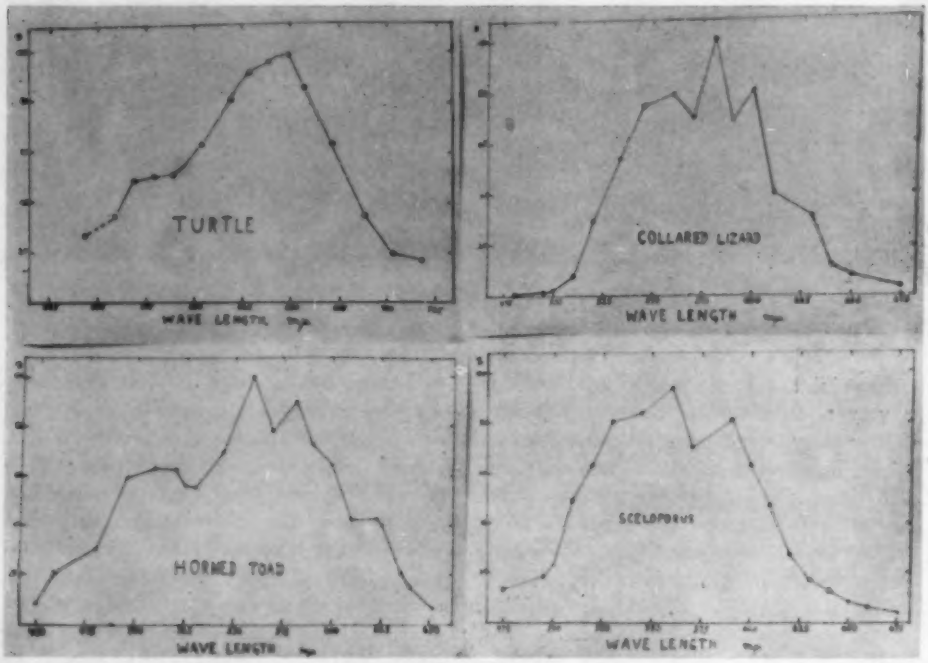

Fig. 12 (MacNichol and Svaetichin). Spectral sensitivity curves of turtle and three species of lizard.

(J. Neurophysiol., **21**:45, fig. 9b, 1958.) The horned toad curve is from a single experiment in which large stable b-waves were found. The collared lizard curve is likewise from a single, fairly typical experiment. The sceloporus curve is an average of three sets of measurements, two of which were successive tests from the same retina.

It is noteworthy that the turtle curve shows the maximum sensitivity in the red (645 mμ) with shoulders in the orange and in the yellow-green. These correspond with the wave lengths at which receptors were inferred from shift-response tests, and also with the oil droplets found in the cones of the turtle in these three colors.

The lizards all show peak sensitivities at about 560 and 580 mμ and smaller maxima between 525 and 550 mμ. These retinas all have yellow oil droplets, about 580 mμ, and colorless ones, but none in the orange or red. The horned toad's sensitivity extends farther into the blue and red than the other two lizards, both of which cut off sharply at about 500 and 650 mμ.

FUORTES: Thank you very much, Dr. Forbes. I think we can now proceed to the rest of the discussion, and the first discussant will be Dr. Frank.

FRANK: I know that you are in a hurry, but I should like to say that the work reported by Dr. MacNichol is very interesting indeed, and I should like to suggest that perhaps the potentials of opposite polarity evoked by different colors might originate by mechanisms similar to those responsible for generation of excitatory and inhibitory synaptic potentials of spinal motoneurones.

FUORTES: I wonder if Dr. MacNichol could postpone a reply.

MACNICHOL: This is very brief. I just wanted to thank Dr. Frank for finishing my talk for me. I was running overtime and did not have time to say that I also think that

our findings are consistent with the notion of two specific transmitters which render the cell selectively permeable to two different ionic species, as it is supposed to happen in ventral horn cells.

The negative-positive responses found in the fish retina appear to be very much like the excitatory and inhibitory postsynaptic potentials described by Eccles in motoneurons. It is entirely possible that each pair of twin cones that were possessed by all of the species of fish described in this report supplied a pair of synaptic endings to single bipolar cells. The green or blue cone would then be expected to discharge a chemical mediator causing a hyperpolarization, and the yellow or red cone a depolarization of the bipolar cell. Our experiments do not contradict such a hypothesis but neither do they prove it.

GRUNDFEST: The work which Dr. Svaetichin and Dr. MacNichol have reported here is of capital importance not only to our concepts of sensory electrophysiology, but also in the general subject matter and theory of bioelectric processes. Since I have been recently somewhat concerned with the latter aspect, I ask your indulgence to discuss these data at some length from that point of view.

One must start with the view that both hyperpolarizing and depolarizing potentials generated by the photic stimulation can evoke spikes in the conductile pathway of the optic system. In his monograph (Acta Physiol. Scand. **39**(Suppl. 134), 1956) Svaetichin remarked that the hyperpolarization of the receptor structures "was accompanied by impulse discharges of the retinal neurons . . . a somewhat unexpected finding which is not compatible with present neurophysiological views" (p. 34).

That statement is no longer valid, since the designation of receptor potentials and of p.s.p.'s as having fundamentally different properties from the electrical activity of axons (Grundfest, H., Physiol. Rev. **37**: 336-361, 1957) can account for these new findings. The data of Svaetichin and MacNichol indicate that the fish retina has a rather complex organization of electrically inexcitable electrogenesis. Presumably, the horizontal and bipolar cells from which they believe the potentials arise are preceded in activity by a primary group of photosensory cells, the cones. Why these produce no potential is puzzling, but since they probably respond with secretory activity, conceivably the latter need not be associated with overt electrogenesis. Absence of an electrical potential despite ionic inequalities across the cell surface has been found by Kao in Fundulus eggs (Kao, C. Y., J. Gen. Physiol., **40**:91-105, 1956). Rather marked independence of the p.s.p. of salivary gland cells from the steady potential of the cell membrane has been reported by Lundberg (Lundberg, A., Nature, **177**:1080-1081, 1956). Other deviations from simple electrochemical relations are also known (Eccles, J. C., "The Physiology of Nerve Cells," Baltimore, Johns Hopkins Press, 1957; Grundfest, H., in "Handbook of Physiology," in press). Thus, it is legitimate to postulate that the cones, at least in the present case, do not develop a potential, but do undergo a photochemically induced secretory activity (Grundfest: Arch. ital. Biol., 1958, in press).

It is necessary to postulate secretory activity because the electrogenesis of the next elements, the horizontal and bipolar cells, is quite clearly electrically inexcitable. This follows from the gradedness of the potentials, from their capacity to last as long as the stimulus does, from the fact that both depolarizing and hyperpolarizing potentials may occur, and from the finding that they appear to occur simultaneously and to summate algebraically. At one point in his presentation, Dr. MacNichol called these postsynaptic potentials. In the definition of p.s.p.'s that I use, these are, indeed, such potentials. However, at no time do these sustained, graded p.s.p.'s produce spikes in the intracellular records. Thus, like some types of electroplax, muscle fibers and gland cells, the cells which generate these retinal poten-

tials lack a conductile electrogenic component. Nevertheless, their activity must in turn affect units with conductile elements, or true neurons. Presumably these are the ganglion cells.

Thus, between the receptor cells—cones—and the conductile neurons there seem to be interposed electrogenic cells of a special variety. They are excited by the photic stimulus, presumably by secretory products of cone activity, and in turn excite, by their own secretory activity, the ganglionic neurons. Their activity therefore resembles rather closely that of gland cells, the secretory activity of which is also associated with a graded, sustained p.s.p. and absence of spikes.

If this is correct, the cells which MacNichol and Svaetichin identify as the horizontal and the bipolar are essentially secretory cells, such as are found in other regions of the brain (Scharrer, E., and Scharrer, B., Rec. Prog. Hormone Res., **10**:183-240, 1954). It is therefore interesting, but essentially irrelevant, that the horizontal cells do not have the appearance of neurons. It would appear that in reality they should be classified as neurosecretory cells. This peculiar arrangement of primary receptor cells, intermediate neurosecretory elements, and ordinary neurons differs from those of receptor structures like the Pacinian corpuscle, or crayfish stretch receptor. In these, the sensory transducer is only the specialized receptor input of a cell which also has an electrically excitable conductile structure, the axon, and which probably terminates in a secretory output membrane (Grundfest, H., Physiol. Rev., **37**:336-361, 1957).

However, at least one other receptor system may be of this same type (Grundfest, H., Arch. ital. Biol., in press*). The hair cells of the organ of Corti impinge upon nerve terminals and may excite the latter by a secretory product. One may also speculate upon the function of the retinula cells of Limulus. Are they merely pigmented satellites of the eccentric cells, or are they the primary photic receptors, affecting the dendrite of the eccentric cell by their secretory products? That they do not appear to develop electrical activity upon illumination seems irrelevant now, since neither Svaetichin and MacNichol nor Tomita (Jap. J. Physiol., **7**:80-85, 1957) find potentials in the cones of the fish retina.

The changes of the responses with different wavelengths of adapting lights also indicate that the potentials are produced in electrically inexcitable electrogenic membranes. A hyperpolarizing electrogenesis in such membrane tends to be diminished when the membrane is hyperpolarized and increased by membrane depolarization. The depolarizing response tends to behave symmetrically, but in the opposite direction; depolarization of the electrogenic membrane diminishes and membrane hyperpolarization enhances the depolarizing responses. This is true for many types of p.s.p.'s (Grundfest, H., Physiol. Rev., **37**:336-361, 1957), the departures from this indicating that special electrochemical conditions supervene. The results with adapting lights reported by Dr. MacNichol thus need signify nothing more than that the responses develop in electrically inexcitable membrane by processes which are controlled purely by passive elec-

* The gustatory cell of the rabbit taste bud also seems to be a specialized receptor, which reacts upon an innervating nerve fiber (de Lorenzo, A. J.: J. Biophys. & Biochem. Cytol., **4**:143-150, 1958) presumably by secretory action. In this junctional system, as well as in the hair cell-acoustic nerve fiber and hair cell-vestibular nerve fiber junctions, "synaptic vesicles" occur in the nerve terminals (that is, they are postsynaptic). It is interesting to note further that of the five great cranial sensory systems only one—the olfactory—which is presumably the more primitive, is mediated by receptors that possess conductile as well as receptive electrogenesis. The auditory, gustatory, vestibular, and visual senses appear to be all mediated by receptor cells which need not have conductile activity, but which transmit excitation to other, conductile cells acting as intermediaries between the latter and the specific stimulus.

trochemical conditions. This is the essence of electrically inexcitable activity. An experimental test of this interpretation is possible. Depolarizations and hyperpolarizations produced by currents applied through an intracellular microelectrode should modify the responses in a predictable manner. With respect to the hyperpolarizing L response which can be only diminished by adapting illumination, because the membrane is then subjected to steady hyperpolarization, the responses evoked by photic stimuli should be increased during membrane depolarization by an applied current.

FUORTES: Thank you very much, Dr. Grundfest, for a very interesting and substantial contribution, not only to our understanding of retinal mechanisms but also to our running overtime. There is a comment by Dr. Wald.

WALD: I am a little mystified as to why spike activity was never recorded during these experiments. I take it that the apparatus would have picked up impulses if firing had been present in any of the retinal layers and I do not understand why the electrode should so frequently land in a "gland cell" of the retina without ever penetrating or approaching a nerve cell. The first interpretation of similar results (Svaetichin, G., Acta Physiol. Scand. **39**: [Suppl. 134], 1956) proposed a new mechanism for color vision. A new interpretation of the same results is more advanced and I wonder whether there are not some accretions of the original presentation that are surviving too long. For instance, I do not see a very clear justification for thinking that these potentials reflect the activity of color receptors. One difficulty is raised by our present lack of information on the relations between these potentials and impulse discharges. One would think that if firing is associated to a potential shift in one direction, potential of opposite polarity would not evoke firing or would suppress firing originally present. I think that the authors have presented an exceedingly interesting set of observations but I think that one should still be cautious in interpreting the relations between the potentials recorded and the various features of color vision. One of the things that bothers me a little is the confident speaking of these diphasic potentials as representing color receptors. Also, one would like to know whether there were rods as well as cones in these retinas as that might change the interpretations quite a bit. I habitually transform spectral sensitivity curves into the spectral absorption curve of some photosensitive pigment and I must say that I'm a little lost here. Since the observations were made on marine fishes, I hardly know how to account for a luminosity function that peaks close to 600 mμ. On the basis of previous observations, one would expect to find a peak close to 550 or 560 mμ. Finally, I wonder whether this color vision argument really receives support from comparison of the observations made with shallow water fishes and deep water fishes. If I understood correctly, the "deep water" fishes are found at a depth of only 30 meters or so and I should like to point out that there is still opportunity to use color vision at that depth.

FUORTES: Thank you very much Dr. Wald. The next is Dr. Linksz.

LINKSZ: At one of the meetings of the Association for Research in Ophthalmology, Dr. Gordon Walls spoke about Dr. Le Gros Clark's work on the lateral geniculate body. As you know, there are double layers of cells in this structure and Clark suggested that they might be a proof for the existence of Helmholtz's three components. I dared to raise my voice and say that in itself this is surely not a proof and that the existence of three double layers could just as well serve as a proof in favor of Hering's theory which also assumes the existence of three types of response mechanisms. And the answer to that was that if one believes in the Hering theory one is almost not worth to be spoken to by fellow scientists. Maybe the

work of Dr. Svaetichin and Dr. MacNichol will make belief in the Hering theory more respectable.*

FUORTES: Thank you very much Dr. Linksz. Two more persons have expressed a wish to make further comments and I shall try to give them an opportunity to do this later. We should proceed now with the fourth communication by Dr. Wagner and Dr. Wolbarsht.

* Dr. Grundfest wishes to add the following remarks:

I wonder if the newly available data don't indicate the need for a reformulation of the fundamental concepts regarding tri- and tetrachromatic theories. These were based on a tacit assumption somewhat like that behind theories of localization of sensation. This assumption is of a direct relation between the causative stimulus and the response of the final effect, whatever and wherever that may be. Various papers of this symposium show quite clearly that the final visual sensory interpretation is a product of many interacting processes, largely neural, including excitatory and inhibitory interactions.

The data involving receptors and secretory products introduce still more complications. From all of these it is attempted to deduce the nature of the photochemical compounds which determine the spectral sensitivities of the primary receptor cells. Svaetichin has argued for the Hering four-component theory, and this seemed plausible when initially he had reported G-R responses that were the reverse of the B-Y potentials. The later data show only smallish, quantitative differences between the two types of responses, both hyperpolarizing at the shorter wavelengths and both depolarizing at the longer. Thus, it is no longer necessary to postulate four qualitatively different color components. For example, different proportions of only two photosensory pigments could be invoked. Differently active secretory and receptive processes might also be postulated. For this reason I suggest that neither theory is disproved by the data and neither is supported by them. The data also seem to indicate that there are probably different kinds of photochemical pigments in different fish. The L responses in different fish peaked at about 450 mμ in one species, 550 mμ in another, and at 600 mμ in a third. In a fourth, the curve of the L response, though also peaking at 600 mμ, was very broad in shape.

STUDIES ON THE FUNCTIONAL ORGANIZATION OF THE VERTEBRATE RETINA*

HENRY G. WAGNER, M.D., AND MYRON L. WOLBARSHT, PH.D.
Bethesda, Maryland

Man has long been curious to learn how his eye can perceive his environment; however, it has only been relatively recently that the retina has been identified as the sensory surface upon which the image of his surround is formed. Since then attention has been directed toward discovering how light could excite the retina. But even though this question has been the subject of intensive study for about 100 years we are still not sure what takes place within the retina. Anatomic studies of the retina have revealed a high degree of cellular and organizational complexity and on the basis of these studies and other work it is now believed that light is absorbed and converted into some sort of neural excitation by the rods and cones of the bacillary layer of the retina. The details of this absorb the efforts of many investigators and much has been learned.

We are concerned in this paper with the subsequent events which occur as the excitation wave traverses the other layers of the retina and arrives at the optic nerve. The importance of the problems involved here transcend the local implications because

* From the Naval Medical Research Institute, National Naval Medical Center. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. Part of the experimental work in this study was done in the Jenkins Laboratory of Biophysics, Johns Hopkins University, Baltimore, supported by Contract Nonr 248 (26) with the Office of Naval Research. Reproduction in whole or in part is permitted for any purpose of the United States Government.

the brain with its structural similarities is likely to possess the same functional mechanisms. For example, elsewhere in this symposium, P. Gouras describes a phenomenon in the toad retina analogous to Leao's spreading depression in the cortex.

The excitation evoked in the bacillary layer traverses at a minimum a bipolar and a ganglion cell before reaching the optic nerve. Numerous accessory nerve cells, long and short horizontal pathways within the retina, and innumerable synaptic junctions have been demonstrated by analytic staining techniques. Figure 1 is a composite drawing illustrating some of the basic architectural features of the amphibian retina with which this paper is particularly concerned. Strong convergence of pathways is another feature implied by the disparity which has been observed in histologic studies between the number of optic nerve fibers and photoreceptive elements. In the frog retina the ratio is at least of the order of one to 30 (Barlow, 1953) and perhaps as high as one to 100. It is reasonable then to suppose that an appreciable area of the image plane is subserved by the average optic nerve fiber. It would be surprising, indeed, if the excitation pattern arriving at the optic nerve was essentially unchanged from the way it began at the photoreceptive layer.

When Adrian and Matthews (1928) disclosed their electrophysiologic findings on the optic nerve of the eel, it was apparent a new and powerful tool was available for studying the functional organization of the retina.

The successful isolation and study of single ganglion cells in the retina of the frog by Hartline (1938) enabled him to announce that the retina was organized into receptive fields and that the ganglion cells

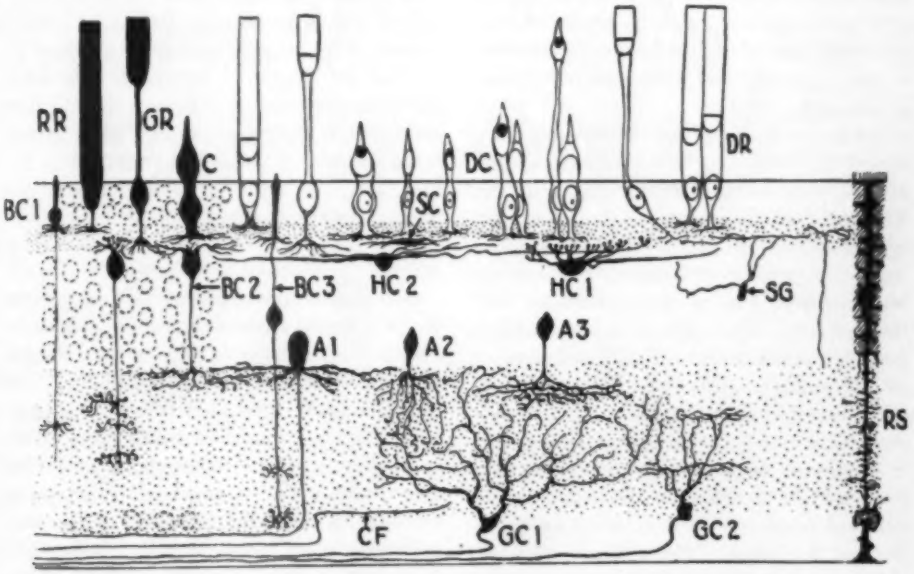

Fig. 1 (Wagner and Wolbarsht). Structure of the amphibian retina (from Detwiler, S. R.: Vertebrate Photoreceptors. New York, Macmillan, 1943). Composite Golgi preparation of the amphibian retina. Redrawn and relabeled from Franz, 1913. RR, red rod; GR, green rod; C, cone; DC, double cone; DR, double rod; BC1, dispersed bipolar cell; SC, supporting cell; HC1, HC2, horizontal ganglion cells; BC2, BC3, bipolar cells; SG, stellar ganglion cell; A1, "nervous" amacrine cell; A2, diffuse amacrine cell; A3, "layered" amacrine cell; GC1, GC2, ganglion cells; RS, radial supporting cell (Müller fiber); CF, centrifugal nerve fiber.

appeared to fall into: "on," "on-off," and "off" type units. This work was soon confirmed by others, and enlarged and extended to other vertebrates thus lending generality to these concepts.

The present studies were begun a number of years ago to amplify and extend these concepts still further.

Method

We followed the technique described by Hartline (1938). The eye of a large bull frog (Rana Catesbiana)* was removed from the animal and the cornea and lens dissected away. Figure 2 shows how a pie-shaped section of the sclera and retina was also cut away to facilitate drainage of the vitreous and allow better access to the optic disc. A small bundle of fibers was teased free from the superficial layer of the retina, divided near the disc, and the distal portion raised onto Ringer-soaked cotton wicks in contact with silver-silver chloride electrodes. Action potentials generated in these small nerve filaments were amplified and displayed oscillographically.

Evidence of single unit activity was then looked for. Often further subdivisions were necessary to obtain satisfactory isolation. Alignment and sharpness of the optical image from the stimulator was determined under a dissecting microscope. The eye was finally sealed into a moist chamber and covered by a light tight box containing a temperature-controlled (18°C.) water jacket. It was not unusual to have these preparations last eight hours. Occasionally preparations were usable for more than 24 hours.

At various times two optical systems have been used in these studies for illumination. One has been described in detail by Hartline and McDonald (1947). It provides two independent optical pathways from a common incandescent filament source. Each pathway includes a neutral density wedge and an electromechanical shutter which together afford a high degree of precision in controlling the intensity and duration of the stimulus. Each beam can project an aperture uniformly filled with light onto the same area in the retina, and from the same direction. These apertures can be varied in size and shape and can be positioned by pairs of crossed micrometers. Provision has been made for externally observing the relative position of the two images. The second optical system is similar in structure to the first, differing only in several details not important in these experiments.

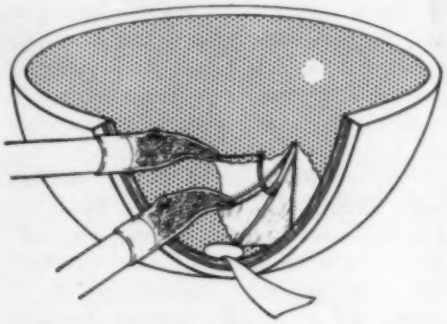

Fig. 2 (Wagner and Wolbarsht). Schematic illustrating technique of isolating single functional optic nerve fibers. (See text.)

Results

A typical response pattern to a long (one second) bright flash of light can be seen in Figure 3. The upper trace illustrates the response pattern described by Hartline (1938) as an "on-off" type fiber. That is, shortly following the stimulation which is indicated by darkening of the open strip within the record, an abrupt onset of repetitive vertical deflections begins; lasts for a few impulses, and then stops even though the light continues to shine. This is the "on" effect. When the light is extinguished there is a similar response with about the same latency including a burst of impulses at about the same frequency which lasts for about the same duration and then stops. This is the so-

* It was observed that if these animals were light adapted for about an hour prior to use less risk of retinal detachment was experienced.

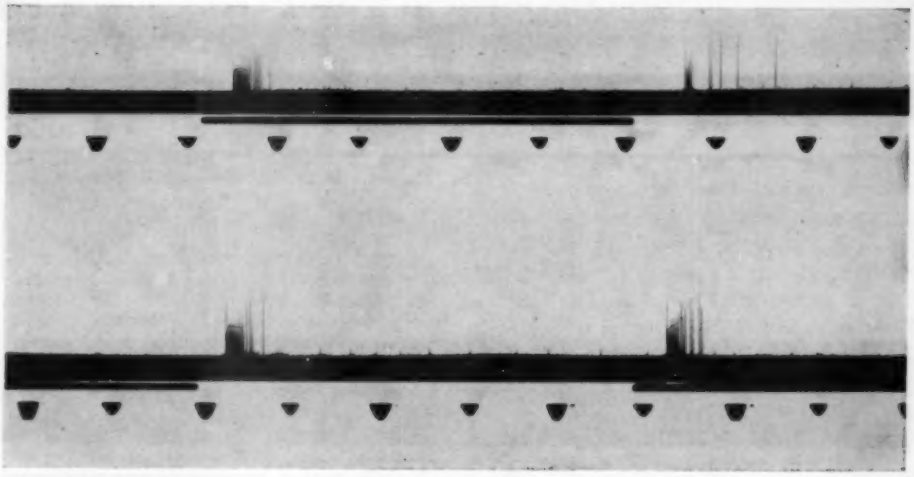

Fig. 3 (Wagner and Wolbarsht). Typical response pattern of an "on-off" type unit. The black band above the time trace indicates the duration of illumination. Time marks at one-fifth-second intervals. Upper record indicates response to a one-second flash of light. Lower record indicates response to a one second interruption of the light.

called "off" effect. Hartline also described a pure "off" fiber in which response is seen only when the light is extinguished and a pure "on" fiber in which impulses continue to be generated as long as the light shines. They are found less often and when found seem to be less stable.

Since "on-off" responses have been found in almost every type of vertebrate and even in an invertebrate eye with such a simple structure as the Limulus (Ratliff and Mueller, 1957), this type of code might well be fundamental to visual system communications. This is further supported by the psychophysical experiments with stabilized retinal images which indicate that to a very large degree only changes in illumination can be seen (Riggs, Ratliff, Cornsweet, and Cornsweet, 1953). It is thus possible that the "on-off" receptor is the most important type in the frog retina. This is the type of receptor that we have selected for all of the experiments reported on here.

The lower record in Figure 3 is from the same fiber. It illustrates the effect of an interruption of light. In this record the first burst of responses is an "off" effect and the subsequent burst is an "on" effect. Since the stimulating conditions are not the same it is not necessary to expect the responses to resemble each other more than superficially. The "on-off" type fiber generally gives an "on-off" type response over a wide variety of stimulating conditions. We have not seen the easy lability described by Kuffler (1953) in the cat where it was fairly easy to convert "on" units into "on-off" units or into "off" units. In spite of the stability generally found, some variations in the response of frog "on-off" units have been seen. This was noticed by Hartline (1938) and also Barlow (1953). Suppression of the "on" or "off" burst can occasionally be produced by adjustment of the intensity and background illumination, but this is unusual. We have not been able to do this at will or predictably.

There is another feature of the response in the frog that deserves mention since it is a constant and troublesome one in trying to make exact measurements. A series of identical flashes will each be followed by a

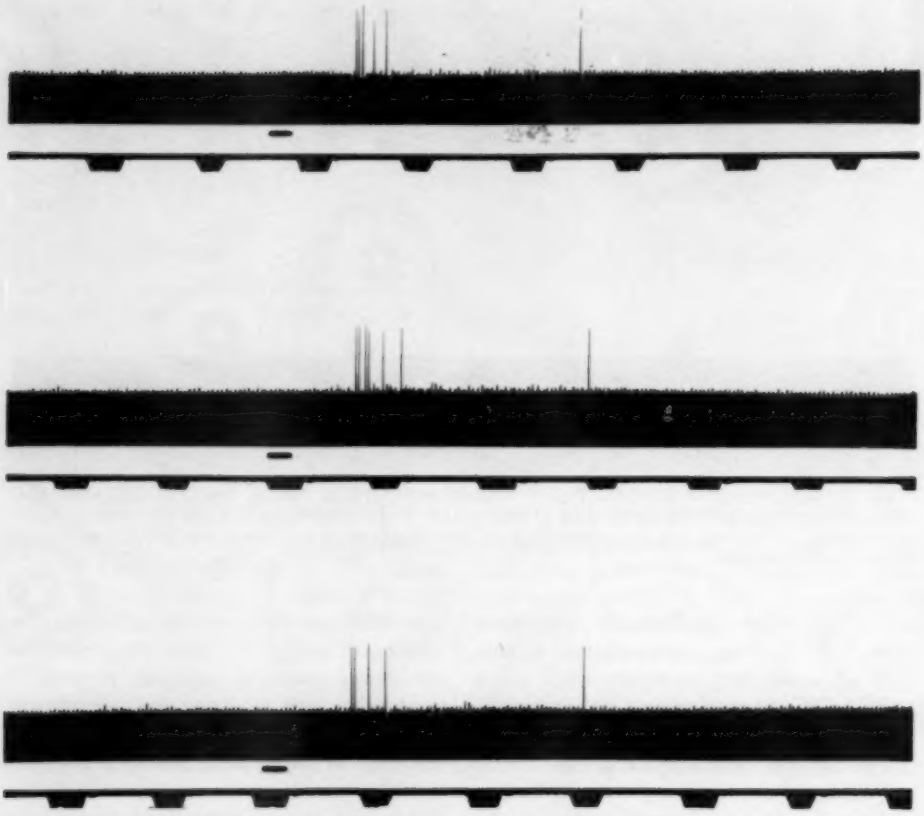

Fig. 4 (Wagner and Wolbarsht). Variations in response to identical stimuli; 0.04 second flash of light. Stimulus and time calibration as in Figure 3.

response pattern that is similar yet congruence is lacking. This effect becomes more conspicuous as the flash duration is reduced to very short values. Figure 4 illustrates the "on-off" response to a series of three identical flashes. The last spike is all that is left of the "off" response. The total number of spikes varies from five to seven, but only the latency of the first and last impulse appears to be maintained with considerable exactness. Scatter is evident in the other spikes. This sloppiness in the response might seem incompatible with the precision that we would associate with such a complex organ as the retina.

When the latencies are plotted as a frequency histograph as in Figure 5, it is immediately apparent that there is a response pattern which may represent an excitation wave in the ganglion cell. This chart was constructed from the data obtained on a different preparation than that shown in Figure 4. The latencies of each spike in the response to a 20 msec. flash of light were accumulated for 95 successive flashes. Each response contained on the average three impulses; however, they varied between two and five. We believe this kind of analysis will warrant further inquiry.

A small spot of light is effective over a large area of the retina in evoking impulses from a single fiber. Hartline (1938) ob-

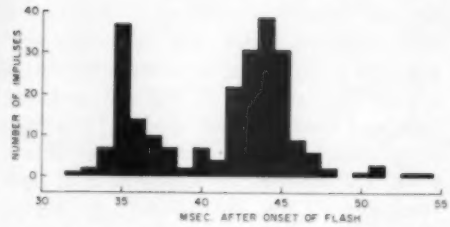

Fig. 5 (Wagner and Wolbarsht). Frequency histogram of the latencies of every impulse in the response burst to each of 95 identical flashes. Each burst contained on the average three impulses; however, as few as two and as many as five occurred in some instances. Duration of flash 20 milliseconds. Ten-second interval between flashes. Intensity of the flash ~0.1 lumens/cm².

served this and termed that area the receptive field of that particular unit.

The responsive area is determined by exploring with a small spot of light of fixed intensity. Moving the stimulus into and out of the responsive area at various places established a series of points as in Figure 6. These points when connected with lines enclose an area inside of which the test flash anywhere will cause a response in the optic nerve fiber but outside of which no response occurs.

Similarly shaped receptive fields have been reported in the cat, rabbit, turtle, snake, and other animals by several investigators. It will be noted that the area is somewhat elliptic. Most of the receptive fields published in the literature are elliptic or irregularly so in outline. Most people, however, are inclined to believe that they are really circular. It is possible that some of the irregularities are real so some attention was devoted to this aspect. An ellipse has been fitted to the test points, the minor axis is 6/10 that of the major axis. This amount of distortion would occur if the plane in which the stimulus moved was inclined about 55 degrees from the plane of the retina. Following the completion of this plot, the alignment of the optical system was checked and found to be tilted at least 45 degrees. A correction for this inclination alone would make the receptive field in Figure 6 nearly circular. From this and other similar experiments we have been convinced that most of the deviations from true circularity seen in the plots are due to distortions introduced by the optical system.

A brighter test stimulus than the one used in this experiment would yield a larger area, concentric with the first. Figure 7 shows the response areas taken at four different brightnesses, spanning three log units. Evidently even more intense illumination would yield larger areas and the central area may be limited by the size of the test stimulus. Scattered light cannot be ignored in all these measurements. A consideration of this aspect is appropriate.

Hartline (1940) measured the brightness of the retina surrounding the illuminated spot and concluded that the halo was at least two log units fainter within a few microns

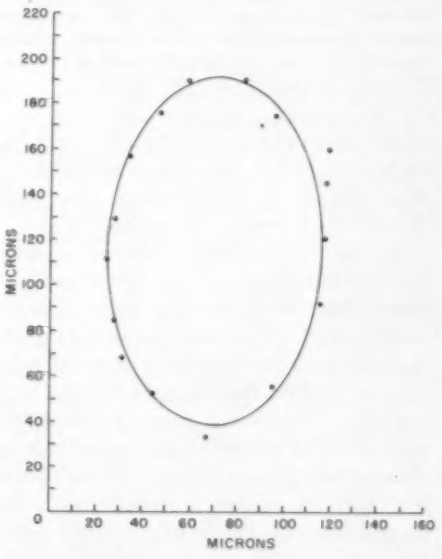

Fig. 6 (Wagner and Wolbarsht). Plot showing the location of points on the retinal surface having a threshold response in an "on-off" type optic nerve fiber when tested with a spot of light of 0.05 mm. diameter, 0.01 second duration, and an intensity of about 0.04 lumens/cm². The solid line is an ellipse having a minor axis 6/10 that of the major axis.

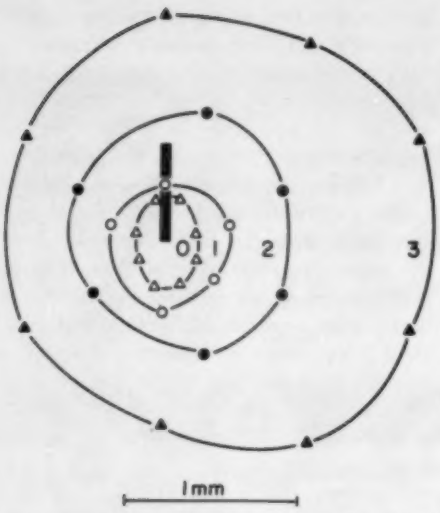

Fig. 7 (Wagner and Wolbarsht). Receptive field plot. The points on the contour lines were determined in the same manner as in Figure 6 for each of four different stimulus intensities. Numbers indicate the logarithms of the relative intensities used. The vertical black line indicates the track along which the determinations in Figure 8 were made. Size of exploring spot was 0.05 mm., 0.01 second duration; 0 intensity = about 160 microlumens/cm².

of the edge of the stimulus. Although his method evaluated the total scatter, he felt that most of the contribution came from Tyndall scatter within the retina.

Barlow (1953) also attempted to ascertain the significance of this effect and concluded that the observed gradient of sensitivity away from the center was more gentle than expected on the basis of scattered light. Another finding that suggests that the large size of the field is not due to scattered light is Hartline's observation that a small moving shadow will cause a response in the peripheral portions of the receptive field. This suggests that the profile of sensitivity across the receptive field may be symbolically represented as something like a cone-shaped figure in which the sensitivity decreases smoothly with distance away from some central point.

We determined the threshold of a tiny spot of light at a series of points leading away from the center. The thresholds were taken at points along a radius depicted as a black line in Figure 7. The same preparation was used for this study. Thresholds were calculated from frequency of seeing plots. The intensity of a long flash of light (one second) was reduced until the region of uncertainity of response was reached. A series of 10 flashes, at each of several intensities, allowed us to plot frequency of seeing against intensity. We found that when these were plotted on probability paper a satisfactory approximation to a straight line was obtained.* Threshold was taken to be the intensity at which a response would occur 50 percent of the time.

Figure 8 shows the actual variation of sensitivity along the track. We found no change in threshold until we were about 250 microns from the center, then the sensitivity began to fall off. That is, we required more light to excite a response. It is also observed that the "on" and "off" responses follow along fairly closely to each other across the receptor field. The observed parallelism is to be interpreted as implying that the "on-off" relationship is not altered in a single flash of light as an aspect of the location of stimulus within the receptive field.

This observation suggested that perhaps the profile of sensitivity could be represented better by a truncated cone or frustum rather than a cone.

We determined the threshold intensities of a series of areas each concentric with the central axis of the same receptive field. In Figure 9 we have plotted the stimulus diameter against the threshold intensity. Each point was obtained in the same manner as on the sensitivity plot just discussed. At least 30 separate observations went into the calculation of each freqency of seeing plot. As the stimulus diameter increased, the intensity necessary to excite decreased until

* C. F. Finney (1952), Page 127 ff., for validity and further details of this approximation.

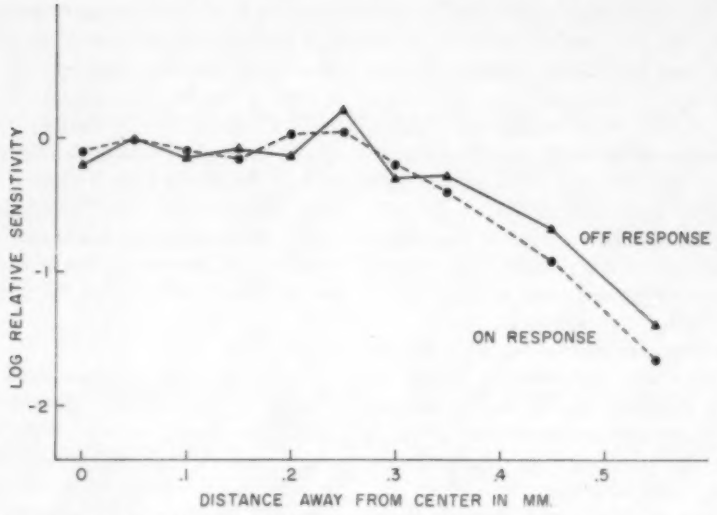

Fig. 8 (Wagner and Wolbarsht). Sensitivity along a radius of the receptive field. Actual track is shown in Figure 7. Test stimulus diameter = 0.05 mm. Flash duration one second; 0 intensity ~ six microlumens/cm². Each point was calculated from frequency of seeing plots as described in the text.

the stimulus diameter exceeded 0.5 mm. This much is in excellent agreement with the previous experiment.

Ricco (1877) described a relationship which subsequently has been called Ricco's law. That is, a constant response should be observed if the total stimulus energy is held constant. The consequence of this is that the product of area, duration, and intensity will be constant for an equivalent response. If there is complete summation on energy basis within the receptive field, the plot should follow Ricco's law and it does out to 0.5 mm.

The data taken with an area of one-mm. diameter required a slightly higher intensity than at 0.5 mm. On the basis of these data alone we cannot attach much significance to this slight rise. We believe, however, that it

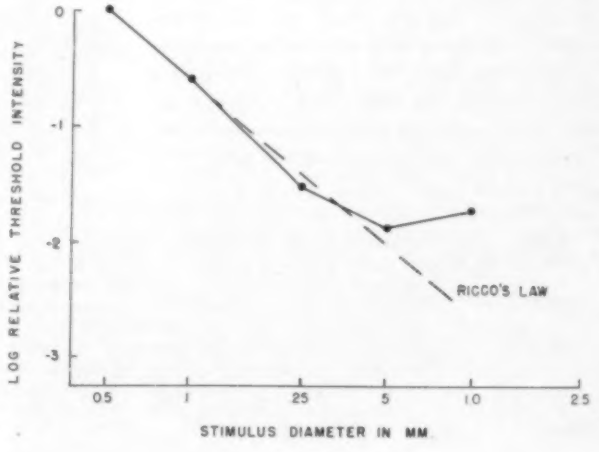

Fig. 9 (Wagner and Wolbarsht). Area-intensity relationship for threshold responses in the same receptive field from which the preceding plots were made. Stimuli were circular, isocentric with each other, and centered at the start of the track in Figure 7. 0 intensity ~ six micro-lumens/cm². Flash duration 0.01 second. Each point was calculated from frequency of seeing plots as described in the text.

is a real rise since other experiments (Barlow, 1953; Wagner and Wolbarsht, 1956) did reveal that peripheral portions of a receptive field have an inhibitory effect on the central area. It is not a strong effect, and is usually demonstrated by illuminating a large annulus of light about a small central spot and showing that illumination of the surround suppressed the response of the central area. Such an inhibitory effect, of course, would cause deviations from Ricco's law in just the fashion that we see.

We believe this agreement with Ricco's law suggests that still another profile of sensitivity should be considered—a circular plateau, or sort of mesa with steep sides. It is obvious that we need more data to resolve this picture more accurately.

These experiments were done in dark-adapted eyes and on the "on-off" type fiber only. We have not observed a change in the size of the receptive field with changes in the state of adaptation of the eye as was reported by Barlow, Fitzhugh and Kuffler (1957) in the cat.

It must not be thought that the response of the ganglion cell is a mere summation of the activity converging on to it from the overlying receptive area. If this were true, as long as a small spot of light were confined within the limits of the central area, the ganglion cell could not distinguish between a stationary and a moving spot. This is decidedly not the case. It is sensitive to movement of the spot. The following experiment will illustrate this point. If two small spots of light are carefully superimposed over some point within the central area of a receptive field and alternately flashed, a suitable intensity adjustment of one of the stimuli will abolish any response. The situation is then analogous to continuous illumination and an "on-off" fiber ordinarily does not respond except to changes in intensity. However, if one of the spots is slightly decentered with respect to the other (10 microns is sufficient), then a response will occur. Neither increasing nor decreasing the intensity of one of the stimuli will abolish the response.

Conclusion

To reconstruct the functional organization of the retina from information contributed by isolated studies of the activity in single neurones is comparable to an attempt to picture a landscape from the impressions collected at night with a small flashlight. Numerous bits of information are being collected concerning the way in which electrical activity in the optic nerve changes with respect to changes in the illumination. Some effort to build these data into a theory of perception has been attempted, particularly with respect to color (Granit, 1947). The functional behavior implied by study of the optic nerve activity may be ascribed to almost any point along the transmission path before this point. Only recently have attempts to intercept information at other points within the vertebrate retina had some degree of success (Svaetichen and MacNichol, 1958).

It is reasonable to expect many new revelations from these studies which may have decisive implications in our thinking on this point.

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Discussion

Ratliff: Both Granit and Hartline have suggested that "on-off" and "off" responses of the vertebrate retina, such as those described by Dr. Wagner, are the result of the interplay of excitatory and inhibitory influences. Recently, Dr. Mueller and I have "synthesized" such responses in the lateral eye of Limulus by utilizing excitatory and inhibitory influences which could be separately controlled. In this eye the inhibitory influences are exerted mutually among the receptors. The first slide (fig. 10) shows some of the quantitative aspects of the inhibitory interaction when only two elements are involved. One can see that the inhibition (decrease in frequency) is a simple linear function of the frequency of firing in the other fiber once a threshold has been reached.

The next slide (fig. 11) shows "on-off" and "off" responses generated by properly balancing the excitatory and inhibitory influences against one another. Now these responses have all of the properties of the analogous responses of the vertebrate eye: the "on-off" responses are characterized by a burst of activity when the light is turned on, no further activity as the light stays on, and a final burst of activity soon after the light is turned off. The "off" responses also have the properties of the vertebrate "off" responses: no discharge appears until the light goes off, and the discharge may be inhibited by re-illumination. I think that these

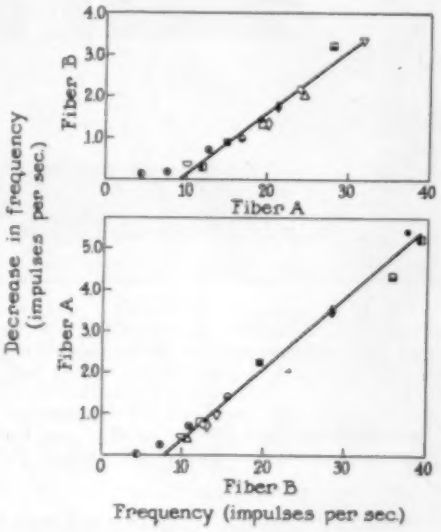

Fig. 10* (Wagner and Wolbarsht). Graphs showing mutual inhibition of two receptor units (designated A and B) one-mm. apart in the lateral eye of Limulus. Action potentials were recorded from two optic nerve fibers simultaneously. In each graph the magnitude of the inhibition (decrease in frequency of impulse discharge) of one of the ommatidia is plotted (ordinate) as a function of the degree of concurrent activity of the other (abscissa). The different points were obtained by using various intensities of illumination on ommatidia A and B, in various combinations. The data for points designated by the same symbol were obtained simultaneously. (From Hartline, H. K., and Ratliff, F.: Inhibitory interaction of receptor units in the eye of the Limulus. J. Gen. Physiol., **40**:357-376, 1957.)

* Figures 10 and 11 were presented by Dr. Ratliff.

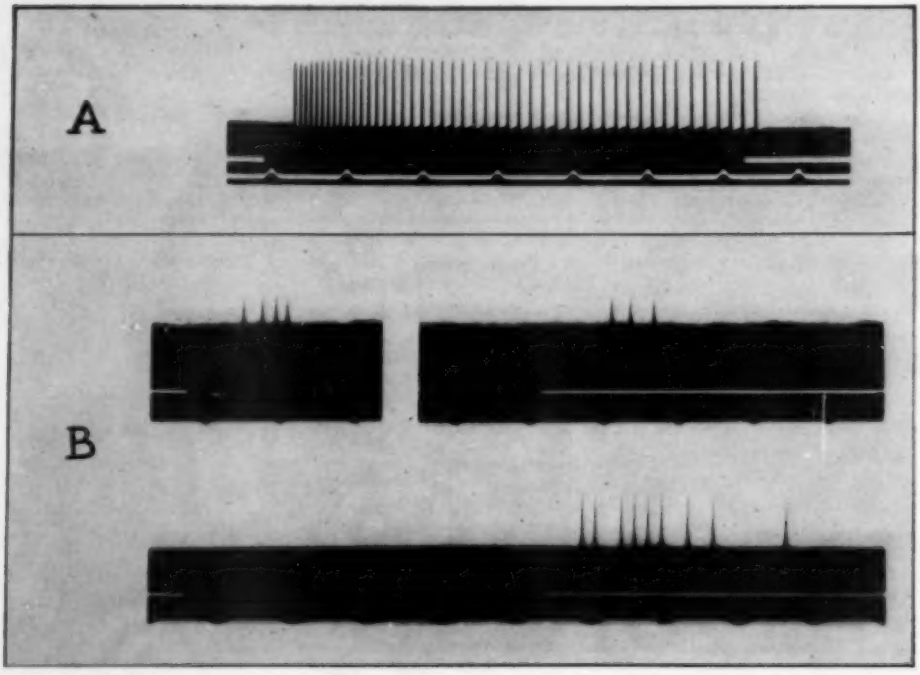

Fig. 11 (Wagner and Wolbarsht). Oscillograms of diverse "types" of impulse discharge patterns in single fibers of Limulus optic nerve. (A) The typical sustained discharge in response to steady illumination. (B) Upper record; a synthetic "on-off" response. (Approximately one second cut from the middle of this record.) Lower record; a synthetic "off" response. Time marked in one-fifth second. Signal of exposure of eye to light blackens white line above time marker. The fibers whose activity is shown in the two B records gave a sustained discharge like that shown in A when the ommatidia from which they arose were illuminated alone. (From Ratliff, F. and Mueller, C. G.: Synthesis of "On-Off" and "Off" responses in a visual-neural system. Science, **126**:840-841, 1957.)

results fit in with Dr. Wagner's description of the organization of the vertebrate retina and offer supporting evidence for the contention of Granit and Hartline that the "on-off" and "off" responses in the vertebrate eye are, in fact, the result of the interplay of inhibitory and excitatory influences.

FUORTES: Thank you very much, Dr. Ratliff. We have now a comment from Dr. Rushton.

RUSHTON: I should like to ask Dr. Wagner whether he was able to confirm the quantitative relation between excitation of the center and inhibition of the periphery that Barlow got about 1950. I am very glad that Dr. Ratliff just made this speech because Barlow's results are contrary to one aspect of the similarity between the beautiful work that they have been doing on Limulus and the rather similar vertebrate work. The observation that I refer to is that Barlow found a linear relation between the light intensity of a spot at the center of a receptive field and the light intensity of a spot at one side, which just inhibited it. That is to say that, if you plot not the log intensity but the linear intensity of light on the center, and found for different intensities here what was the strength of the light on the spot, that there was a linear relation between the two.

Now all the work on Limulus and a certain amount on other things suggests that it is after something like a logarithmic transformation that the linear relations of addition and subtraction occur. I would like to ask whether you were able to confirm Barlow that this relation was a linear one. He did it (I think) over a factor of a thousandfold, so there is a good deal of difference between a linear change and a logarithmic change. If you can confirm that, it would appear to be a very substantial difference between this effect in vertebrates and in Limulus.

FUORTES: Would Dr. Wagner care to reply now?

WAGNER: I have no information to offer on this point.

FUORTES: Are there any more comments to Dr. Wagner's talk? Dr. Hartline.

HARTLINE: I think Dr. Rushton's comment is very interesting and shouldn't go unnoticed. It is certainly a real discrepancy because there is no question that in the Limulus the linear relation of addition and substraction occurs after logarithmic transformation.

FUORTES: Thank you very much. We might resume now the discussion to Dr. MacNichol's paper which we had to interrupt after Dr. Linksz's remarks. Dr. Sjøstrand is the next discussant.

SJØSTRAND: I should like to say that, as observed with the electron microscope, the horizontal cells of the retina present features similar to those of nerve cells while they do not seem to possess the characters which are typical of secretory cells. I suppose, however, that this may be in part a question of definition.

FUORTES: Thank you, Dr. Sjøstrand. The coauthor of the paper will now add a few comments. Dr. Svaetichin.

SVAETICHIN: I certainly agree with all people who have pointed out the difficulties which face interpretation of results such as presented in our paper. However, I have little doubt that the responses described are closely related to color vision. In the first place, as Dr. MacNichol has pointed out, deep water fish present usually only luminosity responses while color responses are often found in shallow water fish.

In the second place, there is good agreement between the results of training experiments performed some time ago by Dr. Grundfest on fish and the results we have obtained. In fact, it is quite remarkable that the sensitivity peaks which have been found in training experiments, making use of colored lights, agree very well with the sensitivity peaks determined by electrophysiologic experimentation.

FUORTES: Thank you very much, Dr. Svaetichin. One more comment from Dr. Kennedy.

KENNEDY: It may be useful to point out that the curves obtained by Dr. Svaetichin and Dr. MacNichol are not spectral sensitivity curves. They are curves relating the amplitude of the potential, and not sensitivity, to wavelength. A further point relevant to the last comment about Grundfest's training experiments is that these were done on fresh-water fish, not marine fish. Furthermore, they were done on a species of fish having a cornea which is markedly yellow, and thus acts as a color filter. A final question concerns structure of the fish retina and nature of the intracellular potentials. If memory serves, there was at first a suggestion that the light responses which reverse their polarity might be attributed to the presence of double cones, which are found quite frequently in the fish retina. I think some recent histologic observations on fish retinas by Lyall are interesting in this connection. It has been shown that there are not only numerous double cones, but often triple and quadruple cones as well. I wonder if Dr. MacNichol or Dr. Svaetichin would care to comment on the influence this kind of histologic arrangement might have on the potentials they have observed.

FUORTES: We are certainly piling up

quite a few questions for Dr. MacNichol and Dr. Svaetichin, and there is a new one now from Dr. Brown.

BROWN: Perhaps I should just mention at this point that Dr. Wiesel and I have recorded certain responses from the cat retina which are very similar to the responses which have just been reported in the fish retina. The similarities are sufficiently numerous and detailed to indicate that the responses we obtain are from single units which serve the same functions in the cat retina as certain of the single units which have been studied in fish. There are, however, interesting differences. Our work along these lines will be given in more detail during our paper this afternoon.

FUORTES: Thank you very much, Dr. Brown. I have a list with the names of the people who have commented on Dr. MacNichols's paper and I can make it available to him, but then he is on his own.

MACNICHOL: First of all, I should like to say that we have speculated about several of the points which have been raised during this discussion. However, we had not anticipated the interesting suggestion made by Dr. Grundfest about the nature of horizontal cells, and I don't know what to say in reply to that. With regard to the effects of electrical polarization, we used a bridge stimulator similar to that used by Dr. Fuortes and Dr. Frank to pass currents through the microelectrode, hoping that we could influence the responses in this manner. For some reason we were unable to change their features with currents up to 10^{-8}A. With regard to the question of why we never recorded spikes, in literally thousands of penetrations we saw small spikes only three times. I don't know what this failure is due to and I want very much to try different types of electrodes, and also Hartline's dissection technique, in order to find out something about impulse discharges in the fish retina.

With regard to Dr. Linksz's comment about Hering's theory. I think Dr. Svaetichin is far more competent to answer this than I am, and maybe there will be an opportunity for him to do so later. I am delighted to hear Dr. Sjøstrand say that these large horizontal cells are nerve cells. It certainly fits in better with the physiologic observations.

FUORTES: Thank you very much, Ted. The cafeteria is open now, but will be closed at half-past one, so there will be just enough time for a quick sandwich.*

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* Dr. Svaetichin wishes to add the following comments:

The basic principle in Hering's theory is that one retinal signal (process) evoked by light of the long-wave half of the spectrum is subtracted from another signal (process) of opposite polarity (direction) evoked by light of the short-wave half of the spectrum. Hering suggested two such opponent color mechanisms; the yellow-blue and the red-green. In addition he proposed a separate black-white mechanism for taking care of the luminosity information. A similar system to the one proposed by Hering would work with four kinds of color receptors, and three would be the minimum possible (Adams-Müller theory). A corresponding subtraction principle in connection with transmission of color information proved to be technically the most economical one in color television.

In the Young-Helmholtz trichromate theory the three color reception mechanisms proposed are also responsible for the luminosity information. Therefore, it was necessary to propose very different sensitivity characteristics of the receptors in order to fit the photopic luminosity curve.

Actually there are certain similarities between the trichromatic theory and the opponent color theory. In any case, the essential differences are not based on the number of different color receptors required. In order to make these statements understandable, we can, for instance, consider the interpretations of red- and green-blindness (dichromatic protanopia or deuteranopia). A red- and green-blind sees the long-wave half of the spectrum in one color (yellow) and the short-wave half in another color (blue) with a neutral gray or white point in the middle at about 500 mμ. According to both theories achromatic gray is created when the two different color reception mechanisms assumed to be present in that case are equally stimulated. Thus, both theories require that the two color reception mechanisms produce equal responses to the wavelength of the neutral point of the dichromatic eye. However, according to Hering the equal but opposite color responses cancel each other at the retinal level and only the luminosity signal is transmitted to the optical centers; whereas, on the basis of the trichromatic

theory, cancellation of signals cannot be assumed since then there would be no signal telling that the neutral point is gray.

Hering emphasized the functional importance for normal vision of the neutral points demonstrated for the dichromate. He proposed that corresponding neutral points exist for the normal eye at the point where the effects of two oponent color reception mechanisms cancel, and that the neutral point is the only region where the normal eye can see a pure hue quality mediated by a third color reception mechanism. The neutral point at about 500 mμ of the red- and green-blind corresponds to the region of pure green quality of the normal eye; the neutral point of the yellow- and blue-blind (tritanope) is at about 570 mμ, which is the region where the normal eye sees pure yellow. It is easily understood from the recordings seen in Figures 5 and 8 how a neutral point for the fish eye could be created when two overlapping spectral response curves are subtracted.

Extensive training experiments on shallow-water fish (v. Frisch, Wolff, Hamburger) proved the existence of a color vision remarkably similar to that of man. Concerning vision of fish, the following has been shown: (a) discrimination ability for 24 narrow-band spectral hues; (b) an ability to distinguish in the extraspectral purple (blue plus red) from any other hue; (c) that white is a sensation basically different from that of hue; (d) the existence of complementary colors similar to those of man; (e) the presence of the intriguing phenomena of simultaneous brightness contrast and simultaneous color contrast (red-green induction, yellow-blue induction). These experimental animal psychology studies constitute an important bridge between the electrophysiologic experiment of the fish retina and the psychophysics of our own vision. Since the vision of man and fish has been proved to be strikingly similar, it is very likely also that the retinal mechanisms for achromatic and chromatic vision are basically similar in man and fish.

It is well known from diving at depths of 30 to 70 meters that the human eye sees the surrounding in a greenish-blue color due to selective absorption of the solar radiations by clear ocean water. The kind of response showing a change of polarity with the change of wavelength was regularly obtained from retinas of shallow-water fish but was never seen in retinas of fish inhabiting depths of 30 to 70 meters. It is hard to believe that so strikingly different spectral response curves would be present just for the purpose of fooling physiologists.

The graded photopic responses (g.p.r.) recorded from fish retinas do offer appropriate, stable signals for a chromatic and achromatic photopic visual system. The nervous transmission mechanisms for the signals from the rods probably are basically different from those of the cones.

ELECTRIC ACTIVITY OF TOAD RETINA*

PETER GOURAS, M.D.
Bethesda, Maryland

Visual electrophysiology has followed two principal lines of investigation which for many years have remained separate, not as much because of a paucity of experimental effort as from limitations in electronic techniques. The earliest approach, discovered independently by both Holmgren[1] and Dewar and McKendrick[2] in the latter 19th century, was concerned with the analyses of the low-frequency electric changes resulting from photic stimulation of the eye. During the subsequent 90 years, this method has not only contributed much information to visual physiology but has also established electroretinography as a useful technique in clinical ophthalmology.

The more recent approach, having developed from improvements in electronic amplification and high frequency recording, began in 1927 with Adrian and Matthews'[3-5] study of the discharge of impulses in the optic nerve of the conger eel. This was extended to an analysis of the activity of single optic nerve fibers of the primitive arthropod eye of Limulus by Hartline and Graham[6] in 1932 and subsequently of the amphibian eye by the former[7] in 1937.

In 1939 Granit and Svaetichin[8] were able to record from the somata of single retinal ganglion cells, developing a technique that has been used by many subsequent investi-

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

gators. Attempts to correlate these two methods of electrophysiologic investigation have been sparse until recently when, with the advent of the high impedance amplifier and the microelectrode, such projects have become more approachable.

In the case of amphibian retina, microelectrode investigations have been reported by Tomita and his co-workers in 1951 [9, 10] and subsequently by Ottoson and Svaetichin,[11] Best,[12] Brindley,[13] and others.[14] With the exception of Best, these studies have been concerned primarily with attempts to determine the origin of the electroretinogram by micromanipulation of the recording electrode to different retinal depths. In this study an attempt has been made to correlate impulse activity recorded on and within the retina with the slow potentials resulting from photic stimulation as well as to analyze their variations with retinal depth in the isolated eye of the toad, Bufo marinus.

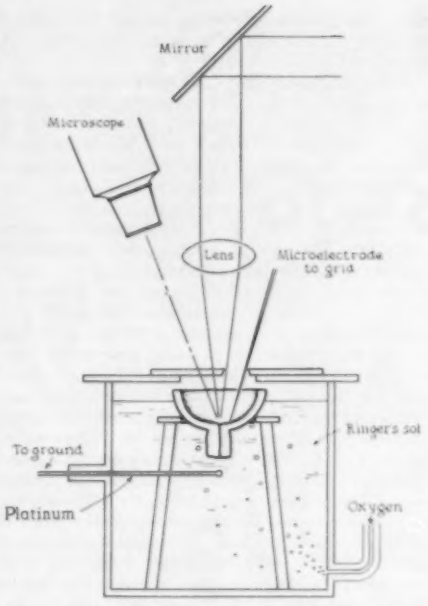

Fig. 1 (Gouras). Diagrammatic sketch of the experimental apparatus.

Methods

The eyes of the toad, Bufo marinus, were used because of their relatively large size compared to most other conveniently available amphibians, the equatorial diameter of the globe ranging from 10 to 15 mm. Although a few experiments were performed on isolated but intact eyes, the routine preparation consisted of an isolated posterior hemispheric segment dissected away from the remainder of the eye along a line approximately at or slightly behind the equator. The retained segment comprised most of the posterior part of the retina, the intraorbital segment of the optic nerve, and the corresponding choroidal and scleral layers.

The lens was removed and as much vitreous as possible carefully absorbed with cotton. Frequntly a small sector was cut out of this segment to facilitate the drainage of vitreous since the sensitivity of the recording electrode was inversely related to the amount of such fluid around its tip.[16, 17]

The dissection was carried out in room light. The preparation was placed into a circular receptacle with the optic nerve down and the posterior scleral surface contacting Ringer's solution in which a platinum electrode was submerged (fig. 1). Oxygen was bubbled into the solution with the outlet being the circular opening on top through which the stimulating light and microelectrode entered. This chamber was attached to a mechanical stage permitting three-demensional movement, thus facilitating both microscopic exploration and focusing of the stimulating light.

The microelectrodes consisted of 3.0 molar KCl filled pipets with outer tip diameters of 3.0 to 5.0 micra and resistances less than 2.0 megohms. Both insulated and noninsulated tungsten wires were also used for electrodes with tips electrochemically sharpened to 2.0 to 3.0 micra in diameter.[18] These proved most adequate for recording retinal action potentials. Larger chlorided silver electrodes were used to measure some of the D.C. changes mentioned in this report and which occurred without changes in the level

of illumination. The electrodes were held by a mechanically driven micromanipulator.

Stimulation consisted of either a bright, diffuse light by which the entire retinal surface could be illuminated or a local light spot, the diameter of which could be changed from 300 to 1,200 micra when focused on the retina by the optical system shown in Figure 1. The source for the bright, diffuse light was a low voltage tungsten filament lamp which could be adjusted to change the retinal brightness from five to 500 foot-candles. This lamp was manually operated.

The light source for focal illumination was either a concentrated arc-lamp or the light resulting from the fluorescence of a P-11 phosphor on the tube face of a cathode ray oscilloscope. Both of these local light stimuli were operated at low frequencies being "on" and "off" for periods of usually five seconds.

The spectral composition of the light from the fluorescent phosphor has a single and relatively sharp peak at 4,600 angstroms. This light source was useful for producing equal energy stimuli of different areas whereas a simple mechanical iris was used for equal intensity stimuli of different areas. The maximum brightness of these local lights was approximately three log units above threshold and could be systematically reduced by the use of neutral density filters.

The entire preparation could be viewed through a dissecting microscope with magnifications of ×6 to ×40, enabling an accurate positioning of the local light spot in relationship to the tip of the recording microelectrode.

The electric potentials were led off from the anterior retinal surface to the grid of a single ended RC coupled preamplifier by the microelectrode and measured relative to the grounded posterior scleral surface. The preamplifier had a tolerable source impedance of 5.0 megohms and permitted variable filtering of the signals from 40 kilocycles to 0.2 cps.

For D.C. changes, a high impedance, unity gain cathode follower was employed at the input stage. Its low grid current minimized the low frequency electrochemical artefacts associated with polarizable electrodes. An ink writing chopper type D.C. amplifier was used to record the very slow electric changes. The higher frequency activity was led off to both a cathode ray oscilloscope and a loud speaker system which permitted listening to, as well as viewing, the electrical signals while they were simultaneously being photographed. The preparation was always maintained at a temperature of 20°C. to 25°C. in a room which was dark except for the rhythmic stimulating light.

Results

Low frequency activity

The amphibian electroretinogram is influenced by many factors including adaptation, stimulation, electrode position, and, in the case of isolated preparations, deterioration.[19, 20] Most responses, however, are variations of a familiar pattern found by the earliest workers in this field and, when recorded with the standard anteriorly placed active electrode, appear as an initial negative wave followed by a positive component at the onset of illumination and a subsequent positive wave at cessation of illumination. These potential changes have been termed respectively the a-, b-, and d-waves. Using direct coupled amplifiers, a positive c-wave of longer duration is recordable during illumination as well as D.C. potentials that are related to changes in the level of illumination.[21]

In the case of the toad eye such classic responses can be recorded from either the corneal or the anterior retinal surface when the preparation is stimulated by relatively bright, diffuse light. These responses are subject to deterioration within an hour or two after dissection in an unoxygenated whole eye but remain longer when the retina is exposed. With the use of oxygen[22] the responses from an exposed retina can be maintained stable for many hours.

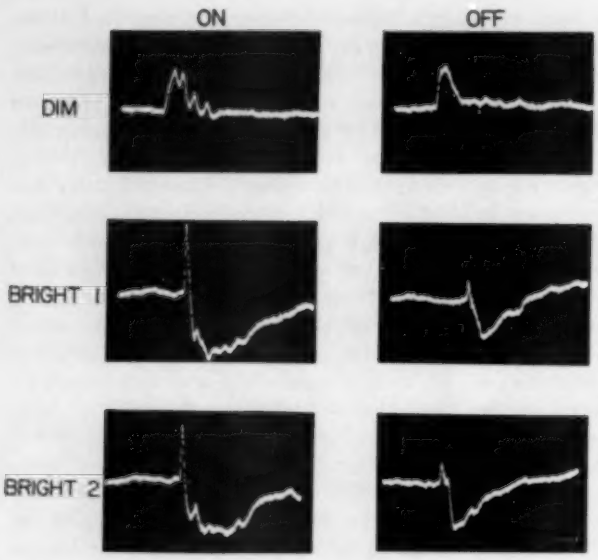

Fig. 2 (Gouras). Oscillograms of the response to dim, local illumination at "on" and "off" (above), immediately after stimulation with a bright, diffuse light (middle), and the second response to this same stimulus 10 seconds later (below). Stimulation begins with oscilloscope trace at dim only. Frequency response is 1.0 kc. to 0.2 cps. Calibration 200 μV vertically and 200 msec. horizontally. Negativity is up.

The responses to focal illumination (300 to 1,200 micra in diameter) are quite localized and if the stimulating light is moved 1.5 to 2.0 mm. from the recording electrode, they become undetectable.[13,14] The responses to low intensity focal illumination are, however, not identical to the classic electroretinogram. At threshold the response to both the onset and cessation of focal illumination is simply a surface negative wave.

Increasing illumination transforms the simple negative responses into the familiar and larger amplitude electroretinogram. If the stimulating parameters are kept constant, these response patterns remain unchanged for hours. The responses differ, however, with changes in the frequency, intensity, or area of the stimulating light but in a predictable and reversible manner.

Figure 2 (above) demonstrates the typical and predominantly negative responses to dim, local illumination. The "on" response to local illumination with slightly suprathreshold stimuli is characteristically sharper and frequently has oscillations in its falling phase. The "off" response may also exhibit oscillations but is usually of lower amplitude than the "on." In Figure 2 (middle) a brighter, diffuse light is suddenly turned on and the familiar electroretinogram appears. The second set of responses to the bright light (Figure 2 below) are not identical to the first, for the amplitude of both components of the "on" response are slightly reduced while the "off" response is increased. If, however, the same pattern of bright light stimulation is continued, the subsequent responses will remain unchanged.

Increases in the amplitude of the "off" response that are dependent upon the intensity of the pre-existing illumination have been reported by Adrian and Matthews,[4] Granit and Riddell,[19] as well as others. With very bright, diffuse illumination the "off" response will become almost purely positive whereas the initial negative component of the "on" response is always retained.

It became of some concern to determine which parameters of the stimulating light produced the positive components in responses which at threshold were entirely negative. Was it the result of an increase in the area or the intensity of illumination or were both these factors important?

In Figure 3 are presented the results from light stimulation of equal energy but different areas, of equal areas but different intensities, and of equal intensities but different areas.

If an equal amount of light energy is distributed over a wider retinal area, oscillations are more prominent whereas if the same amount of light is concentrated, a larger, steeper negative wave of shorter duration and latency occurs followed by a more prominent positive component with less pronounced oscillations.

Increasing the intensity with the area remaining constant tends to increase both the negative and positive components as does simply increasing the area with a constant intensity stimulus, thus indicating that both the intensity and the area of illumination are of importance in producing both components of the electroretinogram. Oscillations in the electroretinogram have been reported by many investigators,[28] the earliest being Frohlich's[24] observations on cephalopod eye.

It is noteworthy that in Adrian and Matthews' work on the conger eel[5] oscillations were most readily produced by diffuse, even illumination rather than by focal light. It can also be seen that within a retinal area 1,200 micra in diameter, area and intensity are not interchangeable as regards to the local electroretinogram. It is also apparent that the familiar electroretinogram resulting from relatively bright illumination represented a gradual transformation of responses which at threshold are purely surface negative. At slightly suprathreshold stimulation a positive component appears. As the stimulation is in-

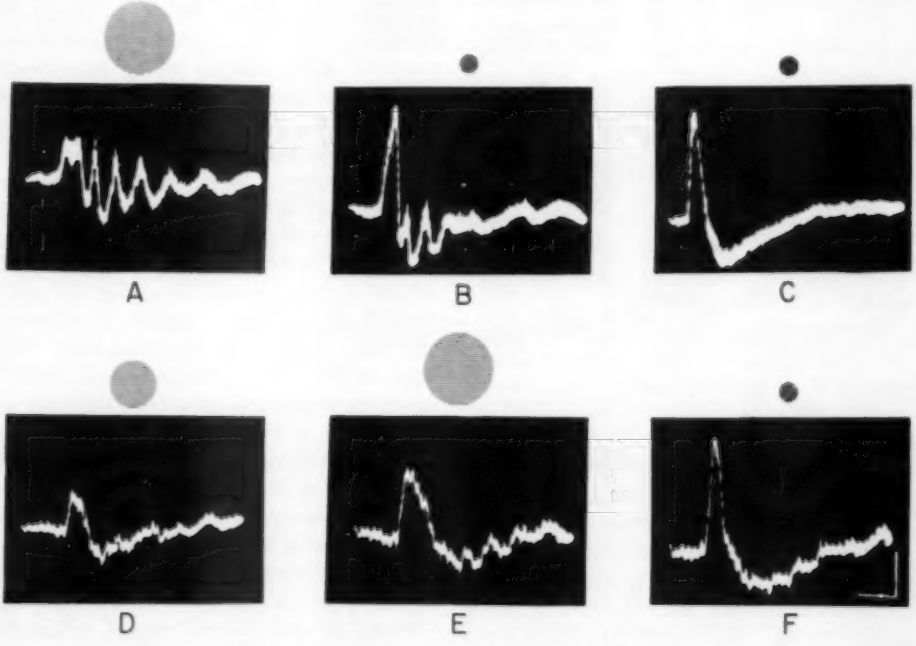

Fig. 3 (Gouras). Oscillograms of "on" responses to local illumination. A and B represent responses from equal energy stimuli but of different areas. B and C from equal area but different intensity stimuli. D and E from equal intensity but different area stimuli, and E and F from equal energy but different area stimuli. The diameter of the large, medium, and small sized spots represent 1,200, 900, and 300 μ, respectively. The relative intensity of stimulation increases with the darkness of the spots. Stimulation begins with the oscilloscope trace. Frequently response is 1.0 kc. to 0.2 cps. Calibration is 50 μV vertically and 100 msec. horizontally. Negativity is up.

creased both these components increase, tending to produce a sharp, short duration initial negative component at "on" with a large subsequent positive wave. At "off" the initial negative wave is never as prominent and with bright illumination is completely supplanted by the positive component.

Ganglion cell activity

With lower resistance microelectrodes and appropriate electrical filtering, action potentials are readily obtainable from the anterior retinal surface. Barlow[10] has shown that these potentials undoubtedly arise from the somata of ganglion cells in contradistinction to the lower amplitude, less constant triphasic axonal impulses.

In this report it has been assumed that these action potentials are led off from the somata of ganglion cells not only on the grounds of Barlow's evidence but also because the patterns of discharge are similar to those recorded by Hartline in single optic nerve fibers,[7] because the responses disappear if the focal illumination is moved 1.5 to 2.0 mm. from the recording electrode, and because they are best recorded 25 to 50 micra below the anterior retinal surface.* It soon became evident that these action potentials were closely related to the local slow waves elicited by focal illumination.

Figure 4 demonstrates this phenomenon. At "on," the initial steep, negative component with a subsequent positive phase is best seen at the highest intensity of stimulation. The corresponding "off" is again of lower amplitude with a more prolonged initial negative phase. Below the slow waves is the high frequency ganglion cell activity recorded at the same locus with identical stimulation. The ganglion cell activity always appears concomitantly with the slow negative waves at both "on" and "off." Reducing the stimulus intensity reduces the amplitude of both the negative and positive components of the slow wave response and tends to make the negative phase more prolonged. At the same time the duration of the ganglion cell activity increases.

In Figure 5, the high frequency activity is recorded simultaneously with the slow waves while the intensity is reduced to slightly above threshold. Except at the lowest intensity, the ganglion cell discharge is more prolonged as the stimulus is reduced whereas its frequency gradually decreases. At threshold the response to both "on" and "off" is simply a surface negative wave with a concomitant low frequency spike discharge.

Depth recording

The purpose of recording photically induced electric activity from different retinal depths is to determine the site of origin of the various components of the electroretinogram by using changes in the polarity or amplitude of an electric response as an indication of the position of its source. Such studies on amphibian retina with microelectrode techniques have been reported by Tomita and his co-workers,[10] Ottoson and Svaetichin,[11] and most recently by Brindley.[13] The problems associated with these techniques are difficult, involving not only electric and mechanical factors but inherent anatomic variations of the retina itself. The depth of the retina exhibits regional differences, deepening gradually from the ora serrata to the posterior pole.[25] As has been shown above, the polarity of the low frequency electric activity also varies with the stimulus parameters which, if not accounted for, can tend to obscure changes recorded at different retinal depths.

Tomita and his co-workers have reported that the electric changes elicited by bright illumination of the entire retina of the bull frog were similar to the electroretinogram when recorded from the anterior retinal surface but became predominantly negative at depths of 70 micra or more within the retina.[10] These changes were entirely reversible

* Measurements on toad retina, fixed in formalin, imbedded in paraffin, and stained with hematoxylin and eosin revealed a depth of 250 ± 50 μ near the pars opticae retinae if a shrinkage of 10 to 20 percent is assumed. The center of the ganglion cell layer is 20 to 30 μ below the anterior retinal surface.

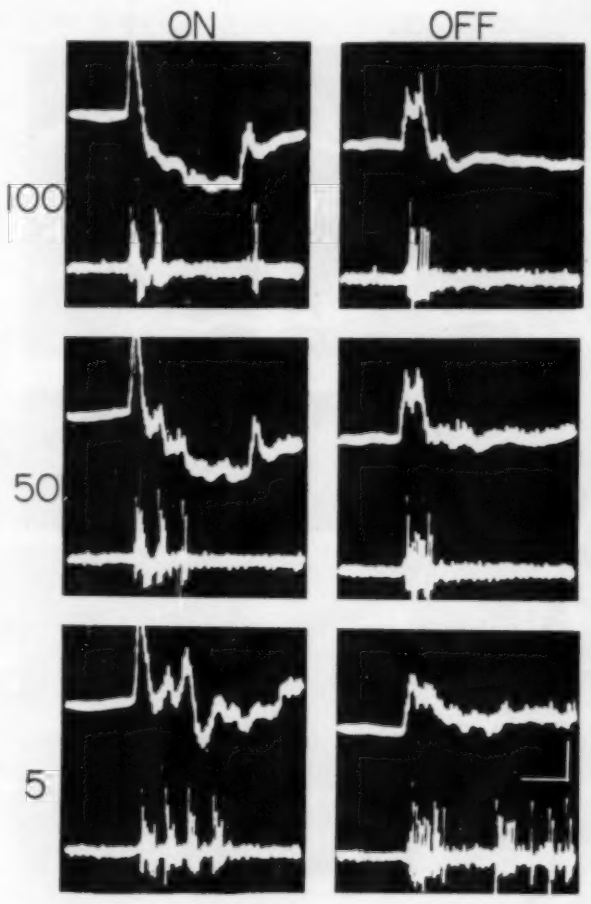

Fig. 4 (Gouras). Oscillograms of the response to local stimulation at both "on" and "off." Intensity is represented in arbitrary units. Stimulation begins with the oscilloscope trace. Above is the response at 1.0 kc. to 0.2 cps. and below at 1.0 kc. to 80 cps. Calibration is 200 μV vertically for low frequency and 50 μV for high frequency responses and 120 msec. horizontally. Negativity is up.

if a 20 micra or smaller electrode was used.

Ottoson and Svaetichin reported that when similar methods were employed on the retina of the frog, Rana temporaria, the electroretinogram did not change polarity, but, at a depth of 150 to 170 micra, rapidly decreased in size without changing configuration.[11]

Brindley, making use of variations in the spatial distribution of the stimulus and its relation to the recording electrode, has somewhat clarified these discrepancies. He noted that the intraretinal response to local illumination was predominantly negative. He postulated that this represented the response of tangentially oriented dipoles, possibly horizontal cells, which could contribute no component to the electroretinogram. The activity of these tangentially oriented dipoles was considered responsible for the disagreements in the intraretinal recordings of the previous authors.

Brindley recorded two response patterns when progresively lowering a microelectrode into the retina from its anterior surface. In one case the electroretinogram remained unchanged until it reached the neighborhood of the external limiting membrane but rapidly disappeared with deeper insertion, thus resembling the results of Ottoson and Svaeti-

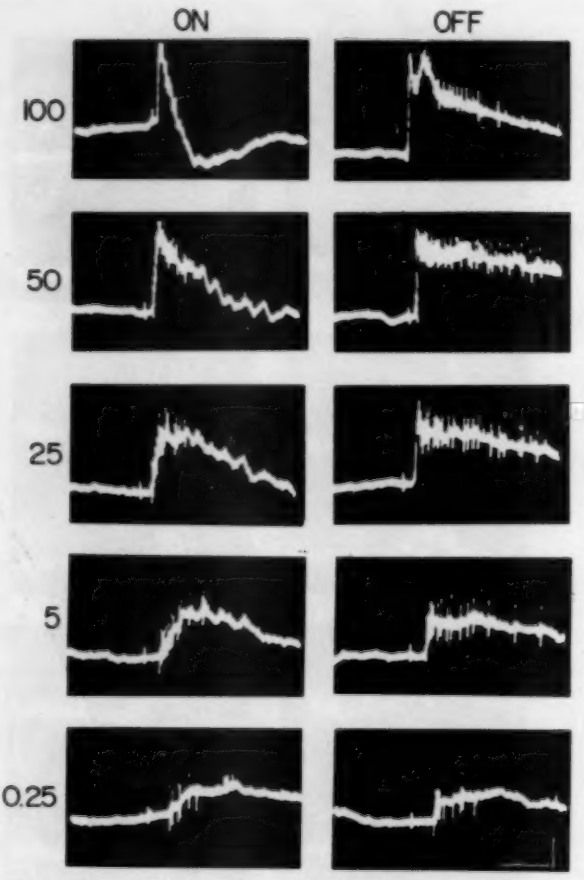

Fig. 5 (Gouras). Oscillograms demonstrating the effect of reducing the intensity of a local stimulus on the low and high frequency responses recorded simultaneously at both "on" and "off." Intensity is represented in arbitrary units. Stimulus noted by artefact in trace. Frequency response is 1.0 kc. to 0.2 cps. Calibration is 100 μV vertically and 100 msec. horizontally. Negativity is up.

chin. In the second case more complex responses were obtained but similar to the results of Tomita.

These patterns were distinguished by the fact that, in the former case, Brindley obtained no response to local illumination whereas, in the latter, the intraretinal negative response was always present. It was this local component which, when still excitable, was believed responsible for the intraretinal negative variations obtained by Tomita. Brindley concluded that the electroretinogram, reflecting only the activity of radially oriented dipoles, was recorded across a region corresponding to the external limiting membrane and undoubtedly represented the photoreceptors.[13]

As has been shown, the response to dim, local illumination is negative at both "on" and "off" when recorded from the anterior retinal surface but that from these threshold responses the familiar electroretinogram progressively develops as either or both the area and intensity of stimulation are increased.

In Figure 6 the data from the most typical depth recording at the posterior part of the retina are shown while alternating the stimulation from a dim, local light to a bright diffuse light at the respective intraretinal depths. The response to local illumination although predominantly negative at the surface demonstrates a positive component at "on."

At 50 to 100 micra, the initial negative component increases in amplitude at both "on" and "off" and spikes become recordable. At this point a later negative component appears that tends to reverse the positive phase of the local "on" response and prolong both the "on" and "off" negative phases. This negative intraretinal component may represent the tangential dipole hypothesized by Brindley. At 250 to 300 micra below the anterior retinal surface the local responses suddenly disappear without changing polarity.

The responses to brighter, diffuse illumination resemble the more familiar electroretinogram with large positive components at both "on" and "off." At 50 to 100 micra below the anterior retinal surface the amplitude of the initial negative waves increases but the subsequent positive phase diminishes. With deeper penetration the later positive phase appears to reverse polarity while the

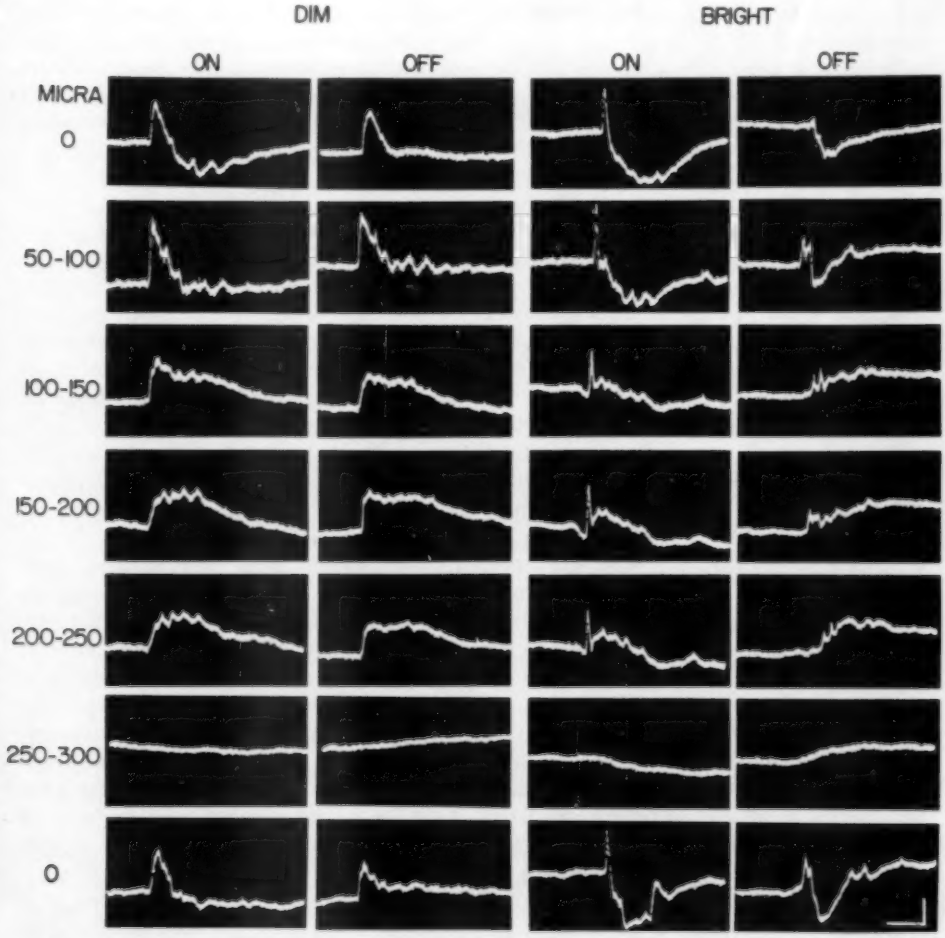

Fig. 6 (Gouras). Oscillograms of the responses at both "on" and "off" from both dim, local, and bright, diffuse stimulation at the indicated depths below the anterior retinal surface. Stimulation begins with the oscilloscope trace at dim only. Frequency response is 1.0 kc. to 0.2 cps. Calibration is 200 μV vertically and 200 msec. horizontally. Negativity is up.

initial negative wave becomes slightly smaller. At 100 to 200 micra an initial positive component is frequently seen but only with bright illumination. At 250 to 300 micra the previous response pattern is lost and a very characteristic low amplitude but long duration response is obtained, positive at "on" and negative at "off." When the microelectrode is withdrawn the responses are seen to be completely reversible although now spikes become more prominent at the surface. This latter change is usually seen after the internal limiting membrane has been punctured.

Spreading depression

A strange phenomenon, that has also been observed by Hartline,[26] periodically occurs and appears to be a pathophysiologic process of excised amphibian retina but which, if one is unaware of, can be extremely troublesome. The process is characterized by a spreading but reversible depression of retinal activity that in many respects resembles the spreading cortical depression of Leão[27] but is unique in that is has an associated color change.

It appears as a misty, grayish wave that will spontaneously occur at the cut retinal edge as early as a half hour after dissection but more frequently as deterioration progresses. It occurs less often with oxygenation or during bright illumination although neither of these two factors are able to prevent its spontaneous appearance. The mist spreads concentrically from its origin at the relatively slow rate of 1.0 to 2.0 mm./minute gradually to traverse the entire retina, although at times it may disappear before reaching the optic disc. Its junction with normal retina is quite sharp and appears to an observer as an intense brown line. As it progresses, the normal color of the retina gradually returns 2.0 or 3.0 mm. behind the advancing wave front.

As this wave approaches an electrode recording impulse activity from the retinal surface, an increase in spontaneous activity occurs which gradually increases into an injury-like discharge as the wave engulfs the electrode. A profound depression follows, so that intense stimulation becomes unable to elicit an electric response. The depression lasts for a period of three to five minutes at which time excitability gradually returns to this local area of retina.

Simultaneous with the arrival of the wave at the recording electrode there is a sudden negative shift of 1.0 to 2.0 mV in the steady retinal potential, which gradually returns in two to three minutes concomitant with the reversal of the color change and the return of retinal excitability. The local low frequency responses to illumination exhibit similar changes but always return slightly before the ganglion cell activity.

Discussion

The data presented reveal that at threshold the low frequency electric changes recorded at the anterior retinal surface are predominantly negative at both "on" and "off" and are closely related to ganglion cell activity. As the light stimulation is increased there is a corresponding decrease in the latency and rising time and an increase in the amplitude of these negative waves which is more marked at "on" than at "off." This is associated with an increased frequency of ganglion cell discharges.

Simultaneous with these changes, however, a later positive component appears tending to shorten the duration of the negative wave and produce the more familiar electroretinogram. This is more obvious at "off" where the smaller negative component of this response may be almost completely supplanted by the positive component with relatively intense stimulation. At the same time the retinal ganglion cells discharge at higher frequencies but for much shorter durations.

There appear, therefore, to be two processes involved at a given state of adaptation, a process contemporaneous with the surface negative waves tending to generate ganglion

cell discharges and a process contemporaneous with the later positive phase associated with cessation of ganglion cell activity.

If these slow waves are identifiable with the amphibian electroretinogram, as seems to be the case, then the a-wave would be contemporaneous with the highest frequency of ganglion cell discharges. Although this view is somewhat different than Granit's interpretation of the a-wave of the electroretinogram,[28] it has some support in the literature.

Adrian and Matthews found that the impulse discharges in the optic nerve of both the conger eel and frog initially increased at "on" and reached their maximum at the same time as the negative component of the retinal response.[3] The fact that Hartline's studies on single amphibian optic nerve fibers revealed that the frequency of "off" unit activity decreased with stimulation six log units above threshold[7] may reflect the completely positive "off" response at this level of illumination.

Depth recording reveals that spike activity is best recorded from 25 to 100 micra below the anterior retinal surface, corroborating the assumption that it represents the discharges of ganglion cell somata. The positive phase of the electroretinogram at the anterior surface tends to become progressively more negative intraretinally whereas the initial negative phase, of greatest amplitude 50 to 100 micra below the anterior retinal surface, does not change polarity. At 250 to 300 micra the characteristic slow wave responses disappear and are replaced by low amplitude, long duration waves, positive at "on" and negative at "off" which may represent photoreceptor[29] or pigment cell activity. The entire process is reversible with these microelectrodes.

The intraretinal negativity that appears during both local and diffuse illumination may represent a tangential dipole or else the negative component of a radial dipole located at 100 to 150 micra below the anterior retinal surface. The initial negative wave of the surface electroretinogram decreases in amplitude at 100 to 200 micra at the same time, however, that an initial positive intraretinal potential appears.

There are two interpretations of these data. One, similar to Brindley's, in which the familiar a-, b-, and d-waves of the electroretinogram are located deep within the retina, at or just above the photoreceptor layer and the intraretinal polarity changes are due to a tangentially oriented dipole. The other postulates two different sources for the negative and positive phases of the electroretinogram, the b- and d-waves most superficial, at 100 to 150 micra below the anterior retinal surface and the a-wave closer to the photoreceptor layer.

Summary

1. At threshold the low frequency electric responses resulting from photic stimulation and recorded from the anterior retinal surface are negative at both "on" and "off" and are quite localized with stimuli of small area.

2. Suprathreshold stimulation tends to produce positive components in both these responses and a gradual transformation into the familiar electroretinogram.

3. Ganglion cell activity is associated with the surface negative waves and absent during the corresponding positive components, suggesting a relationship between the former and generator potentials.

4. Depth recording suggests either the b- and d-waves lie more superficial than the a-wave of the electroretinogram or else all these components originate at a similar locus close to the photoreceptor layer in conjunction with another predominantly negative intraretinal potential.

5. With intense stimulation at 250 to 300 micra below the anterior retinal surface a positive wave is recorded at "on" and a similar negative one at "off," which may represent either pigment cell or photoreceptor activity.

Acknowledgement

I wish to thank Dr. M. G. F. Fuortes for his most helpful advice and for the use of some of his own records.

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Discussion

Lipetz: I should like to comment on the spreading depression described by Dr. Gouras. When he first described it to me, I wondered if it could possibly be associated with an expansion and contraction of the pigment-containing processes of epithelium cells which extend between the outer limbs of rods and cones. But I have later observed a similar change of color and excitability on isolated frog retinas lacking pigment epithelium. I want to mention this because it seems to rule out the pigment epithelium as a

source for the color change. The color change which one sees in isolated retinas must occur either in receptors or in neural elements of the retina.

FUORTES: Thank you very much, Dr. Lipetz. Dr. Grundfest.

GRUNDFEST: The potentials recorded from the vertebrate retina seem to be largely a combination of activities from receptor cells and neurons. Both probably belong to the category of electrically inexcitable responses (Grundfest, H., Physiol. Rev., **37**:336-361, 1957). In that case these must be nonpropagated "standing" potentials, spreading only passively, electrotonically, from the sites at which they are generated. The records of such composite responses depend upon the pathways of activation, the area of active cells involved, and the relative sizes and forms of the component potentials. Changes in shape and amplitude of the overall response therefore are complexly determined and their study must be approached with caution and with the expectation of rather little clear-cut information.

This is illustrated better in the cerebral cortex, where "standing" potentials also predominate in the responses, but where the electrogenic structures are pronouncedly organized in layers and the dimensions of these layers are more favorable for electric pickup. Nevertheless, depth recordings from the cortex are very difficult to interpret, and the difficulty must be many times greater in the case of the retina. One element of the difficulty that needs to be stressed, particularly in the light of the results on fish reported earlier by Dr. MacNichol, is the presence of hyperpolarizing as well as depolarizing "receptor" potentials. I am calling "receptive" those cells which produce graded, sustained electric activity upon exposure of the retina to light. Hyperpolarizing receptor potentials and also hyperpolarizing p.s.p.'s of the neurons may be masked by, or may themselves mask, depolarizing potentials, by algebraic summation. The recorded responses therefore lose a good deal of meaning in terms of underlying events.

One useful tool in cortical electrophysiology is the availability of drugs which inactivate selectively one or the other type of p.s.p. in the dendrites (Purpura, D. P., Girado, M., and Grundfest, H., Proc. Soc. Exper. Biol. & Med., **95**:791-796, 1957). They act not only on cat cortex, but also on bullfrog optic tectum, and might also produce changes in potentials from neurons in the retina and perhaps even in the receptor cells.

FUORTES: Thank you very much, Harry. Dr. MacNichol.

MACNICHOL: I have just one question. A number of years ago, Dr. Wagner and I made some experiments on the frog retina with microelectrodes. If I remember correctly, we obtained spike activity from the ganglion cells and then, about 100 μ deeper, we again recorded spike activity which we thought originated in the bipolar cells. I believe that Tomita described similar findings. Did Dr. Gouras record spike activity from deeper layers in the toad's retina?

FUORTES: Thanks a lot, Ted. Will Dr. Gouras care to reply to the questions and comments?

GOURAS: In answer to Dr. Lipetz's question regarding the source of this color change, I made a few attempts to dissect off the retina from the pigment epithelium as a wave was progressing. This is a difficult procedure and the results were not always clear, but it seemed to me that the pigment epithelium was not essential for the color change. In regard to Dr. Grundfest's comments, I am glad to see that he has drawn an analogy between the retina and the cerebral cortex and admit that interpretation of these summated responses must be cautious. I have not systematically studied the effect of drugs but I have observed effects due to potassium because appreciable amounts of this electrolyte may leak from large pipets. This invariably starts a wave of depression similar to that shown. In regard to the last question of Dr. MacNichol, I have been unable to record

impulse activity below 50 to 100 micra, and the spikes were always largest just below the internal limiting membrane.

FUORTES: I think that this has answered the questions so far, but there is one more question from Dr. Yeandle.

YEANDLE: I was wondering if perhaps the potassium leaking from the pipet might not influence the results. I recently observed that high external potassium abolishes the slow electric potentials generated by the Limulus ommatidium. I was wondering if the leaking potassium might not abolish the slow potentials generated by the photoreceptors.

GOURAS: These changes, however, are quite reversible and always appear at similar retinal depths whether raising or lowering the microelectrode.

YEANDLE: It is also true that if you remove the high potassium from the Limulus ommatidium the slow potential will come back to normal. In your experiments with the toad retina, as you slowly withdrew the electrode from the retina, enough time was provided for the KCl to diffuse away from the photoreceptors so that the potential generated by the photoreceptor could return to normal by the time the electrode had been completely withdrawn.

GOURAS: These changes occur and are reversible with rather sudden shifts of the recording pipet and have been obtained as well with metal microelectrodes.

FUORTES: I think that is a satisfactory reply. Thank you very much for the discussion of this paper.

COMPARISON OF SPECTRAL SENSITIVITY AT THE EYE AND THE OPTIC TECTUM OF THE CHICKEN*

JOHN C. ARMINGTON, PH.D., AND GEORGE H. CRAMPTON, PH.D.
Washington, D.C.

Simultaneous recording from different structures within the visual system has often been used in studies of the chain of events leading from sense cell stimulation to cortical representation. Best[3] and Tomita and Funaishi[13] recorded electroretinographic slow waves and the spike discharge from the retina in an effort to discern their loci and mechanisms of origin. Monnier[12] recorded the electroretinogram and the evoked cortical potential as a means of determining the extent to which the variance of the evoked potential could be accounted for by variance of the response at the sense organ. Lennox and Madsen[10, 11] correlated relative latencies and amplitudes of evoked cortical responses and the electroretinogram to light flashes of varying luminances, durations, and wavelengths, and examined the differential influence of wavelength on the electroretinogram and evoked cortical responses.

The present study is a comparison of the ratios of photopic or cone-initiated activity to scotopic or rod-initiated activity appearing in the evoked optic tectum response and in the electroretinogram under conditions of light and of dark adaptation. Spectral curves were obtained at both locations by means of a data treatment technique that relates sensitivity of fixed response amplitudes to wavelength. Such a technique, although not too common in physiologic studies of visual function, has been of value for the study of the electroretinogram in man[3, 4] and in the chicken.[2] In addition to providing spectral data, this experiment describes some of the physiologic properties of concomitant retinal and tectal responses.

As pointed out in an earlier paper[2] there

* From the Walter Reed Army Institute of Research.

are several reasons for selecting the chicken for study. Its nervous system is dominated by visual structures, and the photochemistry of the eye is relatively well understood, since both cone iodopsin and rod rhodopsin have been isolated from its retina.[14] Thus, the task of interpreting the electrophysiologic data in terms of photopic and scotopic components is simplified.

Experimental method

Rhode Island red hens were immobilized by continuous intravenous infusion of a 1.6 mg./cc. of succinyl choline chloride solution at rate not exceeding 2.0 cc./hr. Intramuscular injection of 0.4 mg. atropine sulfate served to reduce moisture in the respiratory passage, thus keeping the rubber tube from the mechanical respirator clear of obstruction. The lids and nicitating membrane of the test eye were excised, and the skull surface over the contralateral tectum was exposed under local anesthesia (procaine hydrochloride, 2.0 percent). The tectum was exposed by the removal of the skull and caudal pole of the forebrain overlying the recording site. With the succinyl choline infusion rate adjusted to just produce satisfactory paralysis, cloacal temperatures remained at 105 ± 1.0°F. throughout the testing procedure.

The optical stimulator, pictured schematically in Figure 1, was assembled on a lathe bed. Light for the test and adaptation stimulus beams was taken from the front and back of tungsten ribbon filament lamp *T* operated at 2,400°K. The test-beam rays were collimated and passed through a series of interchangeable Wratten neutral density and GAB interference filters *F* to vary their luminance and color. The rays were then brought to focus in the plane of a disc type shutter *DS* set for single flashes of 1/10 second duration. The rays again diverged, but a final lens *L3* made them converge with a visual angle of seven degrees. The bundle of rays entering the eye was 4.0-mm. diameter and of smaller cross-section than the dilated pupil. The adaptation beam was formed in a similar manner. The test and adaptation beams were turned with mirrors *M2* and combined with a beam-splitting cube *B*, so as to present coincident stimulus fields. A camera shutter *S*, permitted exposure of neutral adaptation stimuli when required. The test stimulus when reduced 2.5 logarithmic units below its maximum value with neutral filters matched a white surface having a luminance of 129 ft.-L. The white adaptation field matched a 200 ft.-L. surface. When the 570 mμ interference filter was introduced into the test beam, the test

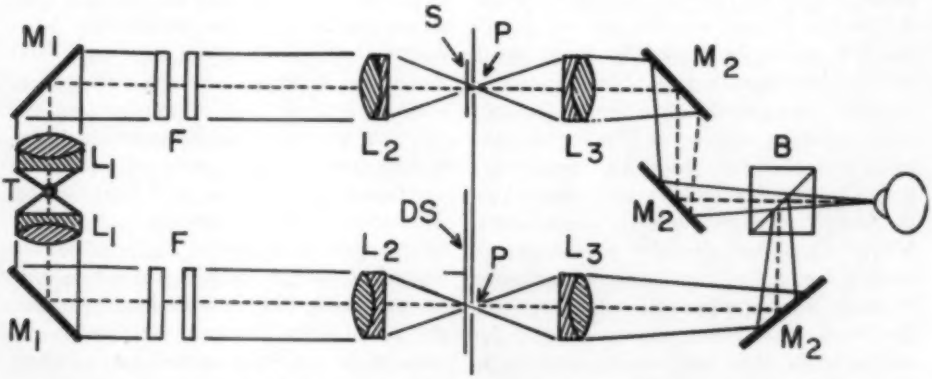

Fig. 1 (Armington and Crampton). Schematic diagram of the optical stimulator. Light for the neutral adaptation (upper beam) and test flashes (lower beam) was combined and focused on the cornea, forming a coincident image smaller than the pupil.

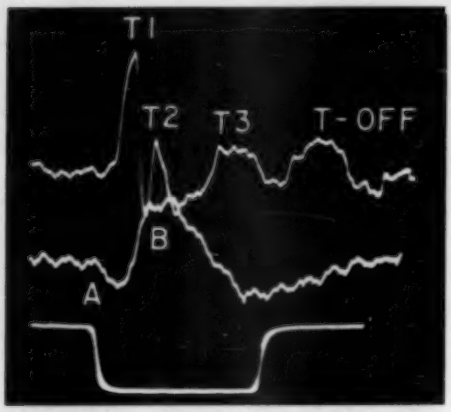

Fig. 2 (Armington and Crampton). Example of responses elicited by a 1/10 second white test flash from the dark-adapted eye. The upper beam shows the various waves in the complex response from the optic tectum. The a- and b-waves are identified in the electroretinogram. Both the b-wave and T1 amplitudes are about 75 μv, with negativity indicated by an upward deflection for the tectal trace and downward for the electroretinographic tracing. Note the prominent off-effect at the tectum when none is apparent at the eye.

luminance was reduced about one logarithmic unit.

The eye was aligned, and the head was firmly mounted on the lathe bed by ear plugs and a beak clamp. Saline soaked cotton-wick electrodes were adjusted on both the cornea of the test eye and the contralateral exposed tectal surface. A common ground electrode for the two pickups was inserted into an incision alongside the comb. The single ended inputs were coupled to each of two Tektronix type 122 preamplifiers; their signals, in turn, were fed to a twin-beam Dumont oscilloscope to be photographed with a Grass kymograph camera. A stimulus mark, activated by the shutter, appeared in all tracings, and a 60 cps signal was used for time measurements. For some procedures, where more accurate timing techniques were required, the tectal and retinal responses were delivered to the same beam of the oscilloscope via a Dumont electronic switch set at a switching rate of one kilocycle per second.

An experimental session was designed to obtain three complete determinations of dark-adapted and light-adapted spectral curves for the retinal and tectal responses. The animal was first dark adapted for five minutes and then light adapted for 10 minutes prior to any recording. Next sufficient test flashes were administered at a variety of luminances and wavelengths to permit a determination of the photopic spectral curve. Forty minutes of dark adaptation followed before the determination of the scotopic spectral curve was made. The three repetitions of this procedure required over 500 test flashes with the flashes spaced at one-minute intervals. Four chickens underwent the complete experimental procedure. Of these, the light-adapted tectal data of one and the dark-adapted retinal data of a second were not complete. A number of additional chickens were tested, but did not undergo the entire procedure because of power line failures and other technical difficulties. Additional white test-flash data, dealing with off-effects and latencies, were obtained in shorter supplementary experimental sessions.

Results

A typical recording is shown in Figure 2. Electroretinograms showed the well known a- and b-wave components. Although these components were actually complex, the tectal responses were yet more so. Changes were sometimes found in the tectal wave form when the stimulus area, wavelength and luminance, the state of the eye's adaptation, and the electrode position were altered. An electrode position could be found, however, as was done in this experiment, where the wave form shown in Figure 2 was obtained. This wave form was simplest in configuration, largest in magnitude, and relatively independent of the stimulus parameters enumerated above. The electrode location was on the dorsal surface, usually near the lateral edge of the convexity of the lobe and about midway between the caudal and rostral poles. This region, lying over the projection area of the central retina,[8] corresponded to the

retinal area upon which the stimulus was imaged. The tectal response was characterized by a prominent negative deflection labeled T1 in Figure 2. With intense test flashes it was followed by more irregular wave forms, T2 and T3. Additional activities of lesser magnitude and regularity which could not be consistently identified occurred throughout the entire period of light stimulation. The tectum produced an off-discharge even with short test flashes, but a retinal off-discharge became apparent only after the termination of a more extended flash duration. Examples of chicken retinal off-effects may be found elsewhere.[6] Additional low amplitude activity persisted for an extended period after the termination of the flash.

As the luminance of the test flash was reduced, all of the components showed a related decrease in amplitude with the a-wave, T2 and T3 components disappearing below the base-line noise sooner than the b-wave and T1. For a given flash luminance the T1 and b-wave responses became larger as the eye was dark-adapted, but T2 tended to be less dependent upon the state of adaptation. Consistent data on T2 were not possible with other than white light, and T3 was never prominent and consistent enough to deal with in a quantitative manner.

The measurement of the electroretinogram was conventional with the a-wave measured from its base-line departure to its greatest negativity, and the b-wave measured from the greatest negativity of the a-wave to the greatest following positivity. The amplitude of T1 was measured from its departure from the base line to its greatest negativity at the peak. T2 measures were taken from the point of reversal of the tracing between T1 and T2 to the negative peak of T2. Figure 3 is a plot of such amplitude measurements as a function of stimulus luminance for white light. As in a prior study,[2] the slope of the b-wave function is greater for the light-adapted than for the dark-adapted condition. A lesser difference in slope is apparent for the tectal responses.

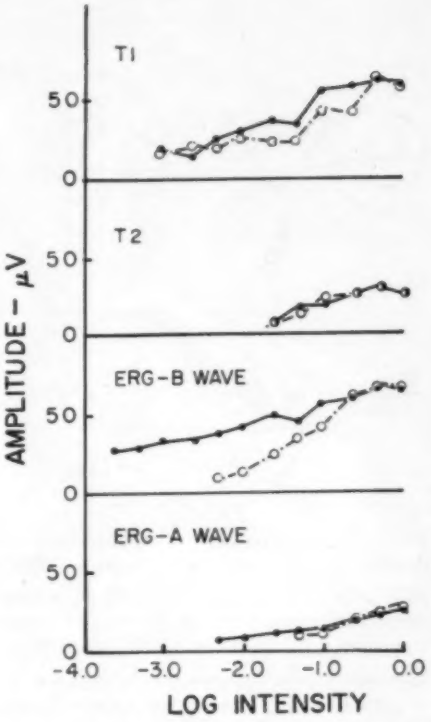

Fig. 3 (Armington and Crampton). Amplitude of response plotted against luminance of white test flashes. The closed and open circles indicate data from the dark- and light-adapted eye respectively. Tectal and electroretinographic responses were recorded simultaneously. The T1 slopes are very nearly the same for the two conditions of adaptation while the b-wave slopes differ markedly.

A brief examination was made of the latencies of the various waves (fig. 4). The interrelationships proved to be very complex and demand a thorough investigation of themselves. Suffice it to say here, that the T1 component arose prior to the onset of the b-wave only with bright flashes and under rather limited conditions. Furthermore, with an appropriate adjustment of test flash luminance, it was possible to have T1 shift from before to after the onset of the b-wave with the shift from dark to light adaptation.

Luminance plots, some of which are shown in Figure 5, were plotted for each color of

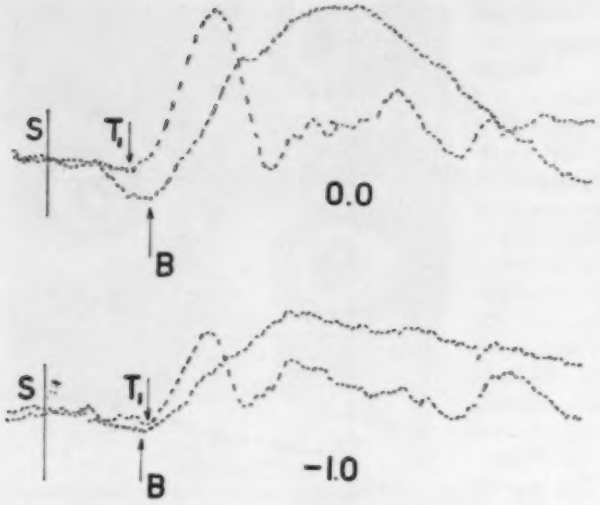

Fig. 4 (Armington and Crampton). Tracings of responses elicited by white test flashes from the dark-adapted eye. An expanded time base has been used. The electronic switch was set at a one kilocycle rate between two tracings. Arrows at the lowest point of each tracing mark the latencies of the T1 and b-waves. The tectum precedes the b-wave for the brighter (0.0) test flash only. S marks the onset of the stimulus flash.

test flash and condition of adaptation. From these plots and with the calibrations of the stimulator, the relative amounts of energy needed to elicit fixed heights of response were computed. A prime purpose of this experiment was to compare the relative spectral sensitivities of T1 and the b-wave. For this reason height criteria were selected so that the sensitivities of each wave could be expressed relative to the same stimulus energy value. This was accomplished by selecting the microvolt amplitude elicited by a 590 mμ test flash reduced 0.6 logarithmic units below the maximum obtainable as a criterion for each wave. Thus, a different criterion height for each wave and each bird was selected, but all spectral results were determined relative to the same test condition. These data, plotted on the basis of a quantum intensity scale, are shown in Figure 6 for two of the chickens. Theoretic curves, described in the discussion, have been drawn between the points.

The chickens did not exhibit Purkinje shifts to the same degree. With Chicken 156, no shift was seen at the retinal level, but there was a clear movement of the spectral peak at the tectum toward the short wavelengths. Chicken 162 showed a shift at both locations; it was, however, more marked at the tectum. The data shown in Figure 6 represent the extreme limits of displacement encountered. The less complete data obtained from other chickens tended to be more like Chicken 156 with the smaller shift at the retina.

It has been pointed out before[2] that the slope of the luminance curve is a function of wavelength and that the electroretinogram has a greater Purkinje shift when the spectral curves are based on low criteria and thus, on low amplitude responses. Figure 7 shows the effect of criterion upon both the retinal and tectal responses. Here the data from Chicken 156 were reworked using a higher (−0.3) and a lower (−1.0) criterion than was employed in Figure 6. It verifies the earlier finding at the retina and shows that it holds at the tectum as well. Nevertheless, within this range of criterion values the tectal spectral curves were somewhat more independent of criterion than was the electroretinogram.

The low amplitudes of the T2 and a-wave components did not permit accurate determinations of their spectral sensitivities. As far as could be ascertained spectral sensivity of the a-wave was similar in form to the

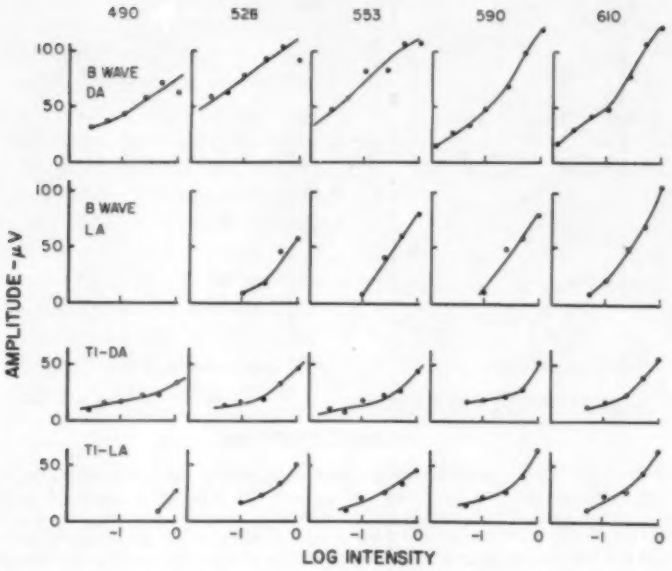

Fig. 5 (Armington and Crampton). Response amplitude plotted against test-flash luminance for several wavelengths. Data are averages of three determinations from Hen 164. Tectal and electroretinographic responses were recorded simultaneously. The systematic variation in slope for the several wavelengths for the dark-adapted b-wave accounts for the various maxima of the spectral sensitivity function when various criteria are used in its computation. This is also true, but to a lesser extent, for the dark-adapted T1. The light-adapted T1 and b-wave each have very nearly the same slopes at all wavelengths.

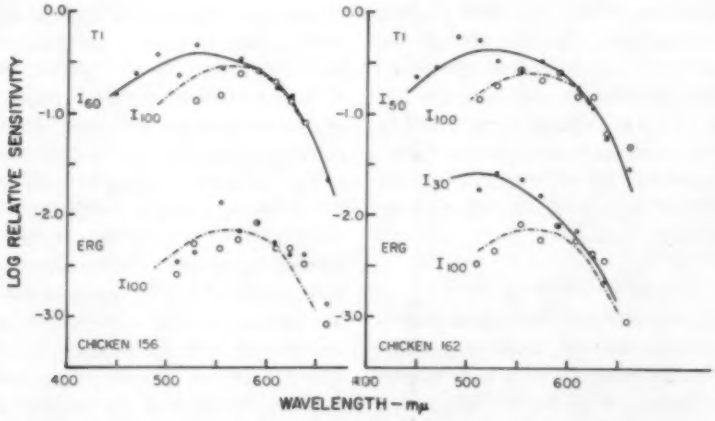

Fig. 6 (Armington and Crampton). Relative sensitivity for two chickens. The data presented here are "quantized." T1 data are arbitrarily displaced from the electroretinographic data to permit easier comparison. The lines drawn through the points were obtained by the combination of the absorption spectra of rhodopsin and iodopsin (see text) that fitted best. A subscript following the letter I indicates the percentage of iodopsin included in the function. The sensitivity of all waves for both hens was obtained on the basis of the response amplitude at -0.6 neutral density filtering with the 590 mμ filter. Note Chicken 156 showed no Purkinje shift at the eye, and yet displayed one at the tectum. Chicken 162, on the other hand, not only had a shift at the tectum, but one of even greater magnitude at the eye.

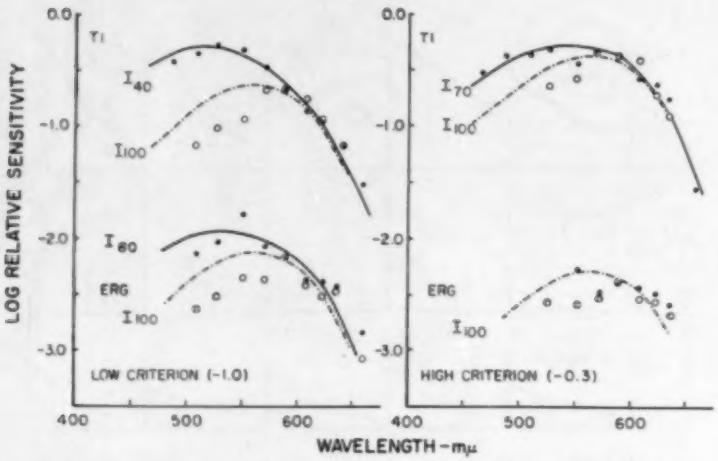

Fig. 7 (Armington and Crampton). Sensitivity data for Chicken 156 reworked for a higher (—0.3) and lower (—1.0) criterion than was employed in Figure 6. The data are presented as in Figure 6. Note that with a low criterion some Purkinje shift can be seen at the eye, although none is seen with the intermediate criterion of Figure 6. With a very high criterion, all scotopic sensitivity peaks moved to the longer wavelengths. A greater shift was shown by the electroretinogram than by the tectum. The retinal response is more variable than that of the tectum.

b-wave, while data for T2 approximated that of T1.

In this experiment as well as an earlier one[2] the greatest variation was experienced with measurements from the dark-adapted retina. This variation does not average out quite as readily as with human electroretinograms where physiologic problems are less formidable.[1, 4] To be certain, there would be less time for variation to occur if test flashes were more closely spaced, but close spacing might result in complicated adaptation effects among successive flashes.

Discussion

Although spectral curves with a variety of maxima were observed, the results do not demonstrate the existence of a wide variety of spectral processes in the chicken retina. To establish this point the assumption was made that the form of the spectral curves is determined for the most part by two photopigments, rhodopsin and iodopsin, both of which actually have been identified in the chicken retina.[14] The further assumption was made that different additive combinations of the pigments were involved in each case. The curves drawn through the points are simple additive combinations of the two pigments which appeared to give a close fit. For example, a curve labeled I_{60} was obtained by the summation, $\log_{10}\ (0.60I_\lambda + 0.40R_\lambda)$. I_λ is the spectral absorption as a function of wavelength for iodopsin, and R_λ is the corresponding function for rhodopsin.

The variability among birds is perplexing. Subjects were fed a supplementary diet of vitamin A in a previous experiment.[2] Although day-to-day as well as hen-to-hen variability was still very much in evidence, the use of criteria that were based on a standard energy test flash aided in comparing spectral curves. Thus, even though it is possible to describe the nature of the differences in sensitivity maxima in terms of proportions of the absorption spectra of rhodopsin and iodopsin, the causes for the differences in relative level and ratio of the pigments are unknown. We may only tentatively conclude that vitamin A level and the

fact that one chicken may have much larger responses than another are not the important factors.

It is perhaps significant that the smallest Purkinje shift is observed at both the tectum and the retina when large amplitude responses are taken as a basis of analysis. Under these conditions, the retina tends to be dominated by the photopic cone components no matter what the state of the eye's adaptation. The reverse is true of the human electroretinogram, where large amplitude responses are dominated by scoptopic activity.[1,4] This may reflect the fact that cones are more numerous in the chicken eye while rods are in the majority in the human retina. A strong test flash produces wide stray light stimulation of the entire retina.[6] This is the condition where responses would be likely to reflect the relative proportions of rods and cones in the entire retina. The tectum is less affected by this factor, however, since substantial Purkinje shifts were seen throughout the range of test-flash luminances employed.

Of particular interest is the fact that regardless of the criterion employed to evaluate sensitivity in the dark-adapted state, there was always a substantial contribution from the iodopsin system. No scotopic sensitivity function reflected rhodopsin alone. This is an interesting divergence from the scotopic data on the pigeon[8] and the chicken[9] collected by behavioral techniques at threshold levels of luminance where there is an excellent correspondence with the rhodopsin absorption curve. Close fits to the rhodopsin spectrum were also obtained from pigeons[7] with the microelectrode technique. This technique requires less light to elicit a response, and samples fewer receptor units than does the electroretinogram. Since larger Purkinje shifts are seen when low criteria are used, it is reasonable to expect that electrical data would agree closely with rhodopsin sensitivity if recording could be conducted at threshold levels.

The closeness of fit of the theoretic curves

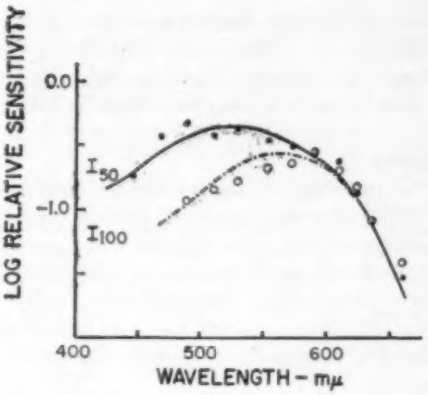

Fig. 8 (Armington and Crampton). Average data for the T1 wave from three chickens.

to an average of T1 data as shown in Figure 8 is noteworthy inasmuch as there is no correction for colored ocular media acting as filtering agents. Colored matter within the lens, at least in the human eye, exerts its greatest effects further in the violet than the present data extend. Hence, if only this source of filtering were considered, the obtained agreement between the tectal data and the photochemical curves would be expected. Another possible source of filtering action lies in the colored oil droplets found within the cones,[10] and it would be expected to exert greatest influence on the photopic (I_{100}) curve. The effect of the oil droplets, however, does not appear to be strong in either the tectal of retinal responses, a finding also true of behavioral measures in the pigeon.[8] To be certain, some of the individual data (Chicken 156) showed a drop in the short wavelengths where the droplets absorb most light (figs. 6 and 7), but this was compensated in the average curve by other individual data which were high in the blue. Small departures from the theoretic curves may possibly be due to oil drops, but it would seem more likely that they result from sources of uncontrolled variability. In order to evaluate the effects of the oil droplets upon gross responses such as the evoked responses of the tectum and the electro-

retinogram, more detailed anatomic information is needed than is now at hand. For example, not all cones have strongly colored droplets, and it is conceivable that their number is not sufficient to influence gross responses.

A number of factors showed that the tectal and retinal responses, although closely associated, still were separate activities:

a. Although the latency of both components was similar, one component could be shifted before or behind the other depending upon adaptation level and test-flash luminance.

b. Off-effects were seen at the tectum for short flashes, but no off-effects appeared at the eye until longer duration test flashes were employed.

c. Volume conduction of potentials from one recording site to the other could not explain the wide varieties of latencies, wave forms, polarities, or amplitudes.

d. Finally, the differing spectral sensitivities of the eye and the tectum clearly indicated that no simple one-to-one relationship was represented.

The complex picture presented by the latencies is of considerable interest. It has been noted that the latency-to-peak of the b-wave is such that it cannot account for much of the early optic nerve activity.[12] Simultaneous recording of the electroretinogram and retinal spike discharges from a sharply restricted area shows clearly that the spikes arise primarily near the end of the a-wave and on the earliest part of the rising slope of the b-wave.[3, 13] These facts appear to eliminate the b-wave from consideration as a necessary link in the arousal of early nerve discharge. The latency data collected incidentally in this study suggest a complexity of events such that the role of the b-wave for evoked potentials is not clear. It can only be said that since the T1 latency can sometimes be shifted from after to before the b-wave latency with appropriate adjustment of stimulus parameters, the recorded b-wave is not a necessary precursor of the first tectal activity. The same argument cannot be made for the entire electroretinogram, however, since the a-wave always precedes optic nerve activity.

Summary

The electroretinogram and evoked potentials from the tectum were recorded simultaneously in chickens. Spectral sensitivity of the two sites was determined from responses obtained over a range of luminances.

1. Purkinje shifts accompanying the change from light to dark adaptation were found at both the eye and the tectum, but the shift was of different magnitudes at the two loci. There was an appreciable variability from animal to animal.

2. Theoretic curves derived from simple additions of the absorption spectra of rhodopsin and iodopsin were successfully fitted to each of the various spectral curves. A correction for the filtering effects of the colored oil droplets was not required.

3. All theoretic curves fitting the scotopic data required certain amounts of iodopsin in addition to the rhodopsin. If spectral sensitivity was derived from data employing high luminance test flashes, more iodopsin was required than if the sensitivity was determined from low luminance test flashes.

The technical assistance of Nathaniel Boggs, Jr., is gratefully acknowledged.

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Discussion

FUORTES: Dr. Kennedy would like to comment on this paper.

KENNEDY: I wish to make two comments. The first concerns the latency measurement of the tectal response T1 and of the b-wave. Measurement of the latency of the b-wave is difficult because the b-wave is immediately preceded by the a-wave, which has possibly a different source and certainly an opposite polarity. When two interacting potentials are present, I do not think that one can place too great confidence in the measurement of the latency of the second, since the magnitude of the first is going to affect the apparent latency of the second. The second point I wished to raise concerns absorption in cone oil droplets. The sensitivity curves presented by Dr. Armington show a maximum near 600 mμ, whereas the absorption maximum of iodopsin is, as Professor Wald has shown, around 560 mμ. It seems to me that close scrutiny of Dr. Armington's data suggests that a correction is necessary.

ARMINGTON: I think it is true that measurement of the b-wave latency is complicated by the fact that the a-wave comes first. There are a number of other things that complicate this type of measurement. Hence, showing that the b-wave may either precede or follow a tectal response is perhaps only of academic interest. The experiment reported here shows that the ratio of scotopic to photopic activity is different at the eye and the tectum. Thus, it is possible to select conditions where the latency of a photopic retinal response is compared with a scotopic tectal response. We recognize this possibility in the present experiment, and it needs to be considered when interpreting the results of similar experiments. The role of stray light, particularly for the electroretinogram, also has to be considered. If the eye is dark-adapted and a bright test light is used, the electroretinogram apparently comes from the entire retina, which is stimulated diffusely and not very intensely by stray light. This can produce a long latency b-wave. Latency of the tectal response may be less influenced by stray light, but this has not been worked out yet. Despite all these limitations, Monnier and other investigators have found b-wave latency measures to be of considerable value.

There may be some small evidence of the oil droplets, but it is not very striking. The transmission of the oil drops is such that one would expect a reduction in sensitivity in the yellow-green, particularly below 550 mμ. The data shown for one of the chickens did have such an effect. Other hens had a higher sensitivity in this region, however. When the results from several hens were averaged a clear-cut depression below 550

mμ was not seen. The transmission of the oil drops may vary with the season of the year and with diet. These hens were all tested in the spring. Perhaps a different diet or a different time of the year would enhance the droplet effect.

FUORTES: Thank you very much, John. Dr. Rushton would like to ask a question of Dr. Armington.

RUSHTON: You had theoretic curves of rhodopsin and iodopsin added in various proportions. As you plotted them the curves were on a logarithmic basis and I would like to ask you whether the addition was a certain fraction of log rhodopsin absorption added to log iodopsin or whether it was from a linear basis. Whichever it was, what do you suppose is the justification for the addition?

ARMINGTON: The addition was on the basis of the linear scale. That is, the photochemical data were expressed in linear units and added. Then the logarithm was taken. The question of justification is a complicated one. Perhaps it would be best to say that this was the simplest thing and it worked. This technique has been used by other investigators when mixtures of pigments were involved. I believe some of Dr. Wald's papers may have influenced us a little bit when we adopted this procedure.

FUORTES: It has been suggested that it might be interesting for part of the audience to go back to invertebrates. Dr. Yeandle has not had an opportunity to show some data he has recently obtained on Limulus and perhaps we could convince him to come to the microphone now. Dr. Yeandle.

YEANDLE: This morning there was discussion about the relationship of the slow potential to the production of action potentials in the Limulus ommatidium. What I have to say has bearing on this problem. But before talking about my own work I would like to discuss the problem of determining the number of quanta necessary to be absorbed in order to produce a visual response.

Ever since the classic work of Hecht, Shlaer, and Pirenne (1942), in which it was shown that very few quanta are necessary for perception, the exact number has been controversial. These authors used two main methods for determining the absolute threshold of vision. In method one, they accurately determined the average number of quanta at the surface of the cornea in a pulse of light that was just at the threshold for perception, estimated the percentage of light removed by the structures of the eye between the cornea and the retina, and calculated the optical density of the visual pigment from rough estimates of its concentration and a knowledge of its extinction coefficient. From these considerations the average number of quanta absorbed by the retina when a known number of quanta was presented to the cornea could be estimated.

Method two is based on the impossibility of designing an apparatus that will produce a pulse of light which always has exactly the same number of quanta. Only the average number of quanta can be fixed in a light pulse. It can be shown that, if a is the average number of quanta in a pulse of light and p is the probability that a quantum incident on the surface of the eye will be absorbed and lead to a physiologic response, then the probability, G, that y, or more quanta are absorbed and lead to a response is

$$G(y, pa) = 1 - \sum_{r=0}^{r=y-1} \frac{e^{-pa}(pa)^r}{r!}$$

A response is defined as any type of event that can be observed unmistakably. Pirenne (1950) has given an excellent discussion in *Progress in Biophysics* as to the implicit assumptions made in using this equation for determining quantal requirements for a response.

It is more convenient to consider the probability of response as a function of y and log pa rather than y and pa since the factor, p, is generally unknown and, usually, only the relative average number of quanta can be determined by use of an optical wedge.

That is to say, if the average number of quanta per flash is equal kI, I can be determined by an optical wedge but k is unknown. However, if one plots the probability of response as a function of log I, the factors k and p do not affect the shape of the curve, but only displace it along the log I axis. Hecht has drawn the theoretical curves of probability of response versus log a for different values of y so that, to determine the quantal requirement for a response, it is necessary to observe which theoretical curve best fits the experimentally determined frequency of response versus log I.

Using the methods developed by Hecht, and other statistical methods which will not be discussed here since they are not useful in the present study, various workers have found the number of quanta necessary for perception in the human to be anywhere from two to 32. The controversial literature associated with this work is excellently reviewed by Pirenne (1950) and Weale (1955).

In the hope that a system simpler than a whole organism might give more definite results, Hartline, Milne, and Wagman (1947) studied the number of quanta necessary to produce an action potential in Limulus. Using method two these authors found that the number of quanta required to produce an action potential was a small number. Furthermore, more quanta appeared to be necessary to produce an action potential in the light-adapted eye than in the dark-adapted eye. There was considerable variation among preparations which had been completely dark adapted as to the quantal requirements for an action potential. This variation is difficult to explain but perhaps the present work may help to provide an answer to this problem.

Using internal micropipettes, Hartline, Wagner, and MacNichol (1952) observed, in the Limulus ommatidium, slow depolarizations that were induced by light. These authors presented evidence for the hypothesis that these slow depolarizations generated the action potentials discharged by the ommatidium. If this is true, one should expect the slow potential to be roughly quantized, or, in any case, to vary statistically in size in response to a constant light pulse that is near threshold for the production of a slow potential. MacNichol has found suggestions of such an effect but has made no attempt at a statistical analysis.

Two types of responses were found in the ommatidium, presumably depending on the exact location of the microelectrode. In the first type, large action potentials (20 to 40 mV) were seen superimposed upon a much smaller slow electrical potential. In the second type, 1.0 to 5.0 mV spikes were seen superimposed on rather large slow potentials. Because dense melanin pigment is present in the retinula cells it is impossible to observe visually the exact location of the microelectrode. In random puncturing of Limulus ommatidia the second type is seen much more frequently than the first type. Since less area is presented to the microelectrode by the eccentric cell than by the retinula cells and the rhabdom, it is tempting to make the hypothesis that the first type of response is recorded from eccentric cells while the second type is recorded from retinula cells or the rhabdom. A technique is now being developed to localize the electrode and until this is developed one can only guess where the electrode is in any given experiment.

Occasionally, it is possible to see small quantized slow potentials in preparations of both types. Figure 9 shows records from a preparation of the first type where the electrode was probably in an eccentric cell. Throughout this experiment the eye was in the dark except when test light pulses were presented. The duration of pulses was always the same (30 milliseconds), but the intensity was varied. After a pulse of given intensity, a response sometimes did and sometimes did not occur. The response seemed to consist of quantized slow potentials which, if they summated to a certain height, caused an action potential. For lack

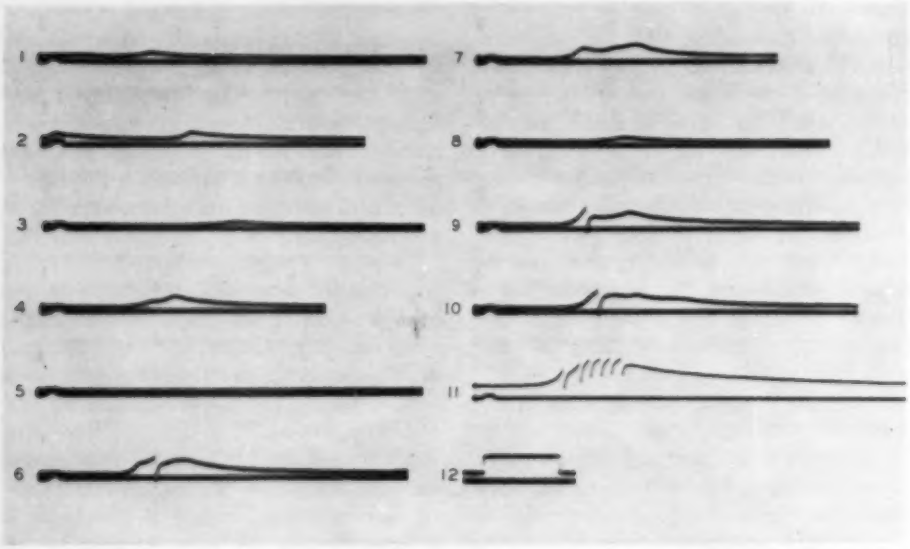

Fig. 9* (Armington and Crampton). Experiment of the type showing large action potentials. Duration of light pulse in all pictures is 20 milliseconds but the intensity in each picture is different as shown below:

Picture	—Log intensity of light	Picture	—Log intensity of light
1	5.00	7	4.50
2	5.00	8	4.50
3	5.00	9	4.00
4	5.00	10	4.00
5	5.00	11	2.00
6	4.50		

Picture 12 is a 10 mV calibration applicable to all pictures. Step in bottom trace shows when light went on. Small pips on bottom trace are spaced at 10 millisecond intervals.

of a better term these quantized slow potentials will be referred to as bumps. Occasionally a spontaneous response would occur which could not be associated with the light flash. The first bump in picture 2 of Figure 9 occurred exactly when the light went on and is probably not associated with the light. If more than one bump occurred after a pulse of light, the latencies of the bumps were not all the same.

In pictures 1, 2, and 3 of Figure 9 only one bump was elicited by the light. These bumps were not all the same size. This may mean that although each bump was the result of one quantized action, each bump originated in a different part of the ommatidium. Since the bumps were probably attentuated at the microelectrode and the amount of attenuation very likely depended on the distance between the microelectrode and the place where the bump originated, the height of a bump would depend on where it originated.

Although the intensity of the light in picture 5 (Figure 9) was the same as in pictures 1, 2, 3, and 4, no response occurred. In picture 4 it appeared that two quantized bumps occurred, and in picture 7 three bumps occurred. In pictures 6, 9, and 10 enough bumps occurred at the same time for the slow potential to rise high enough to cause an action potential. In picture 11 the

* Figures 9 through 11 were presented by Dr. Yeandle.

light was sufficiently bright to cause the slow potential to rise high enough to produce six action potentials. In this experiment not enough data were gathered to do Poisson statistics to determine whether one quantum of light was associated with one quantized bump. Yet the appearance of a bump after a flash of light is statistical in nature and may be due to one quantum.

Figure 10 shows records from a preparation of the second type where the electrode is probably in a retinula cell or the rhabdom. The character of the bumps is different here than in the preceding experiment. In this experiment the eye was kept in the dark except when test pulses of light, all of the same intensity and duration, were presented to it. Occasionally, spontaneous bumps would occur which could not be associated with a light pulse. Peculiar spikes would occur, superimposed on the bumps, but these spikes did not look like ordinary action potentials. Pictures 1 and 2 of Figure 10 show these spikes which are probably eccentric cell action potentials whose heights are markedly attenuated since the electrode is probably located some distance from the origin of the action potentials.

As in the preceding experiment the number of bumps per test flash was variable. Pictures 2 and 5 of Figure 10 show more than one bump. Pictures 3, 4, 6, and 8 of Figure 10 show only one bump, yet the size of the bump is extremely variable. Tentatively assuming that one bump represents one quantized action, one might explain the variability in size of single bumps by the same mechanism used to explain a similar effect in the preceding experiment. However, in this experiment the variability is much greater. The explanation for this might be as follows:

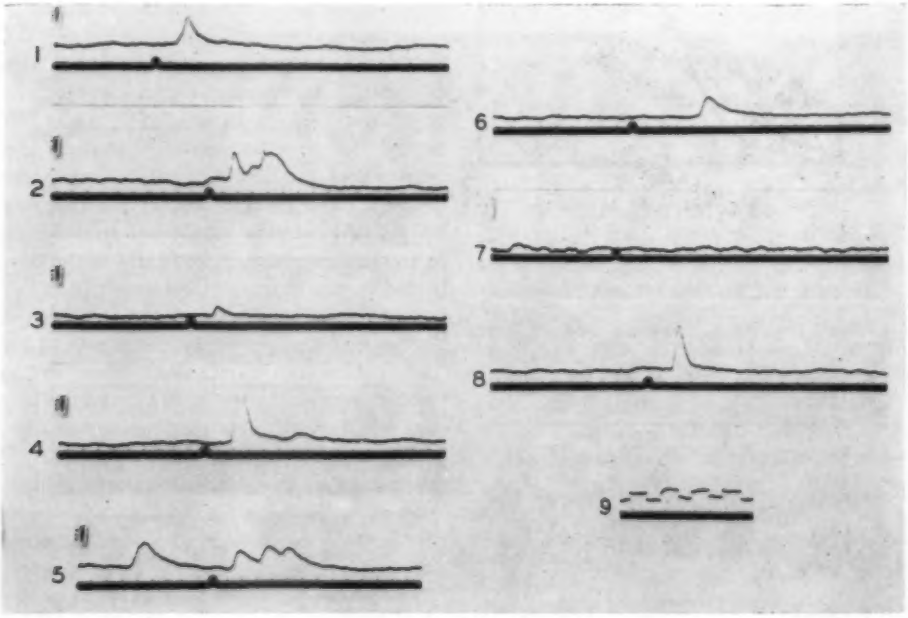

Fig. 10 (Armington and Crampton). Experiment of the type showing small spikes superimposed on slow potential. In all pictures the duration of the light pulse is 30 milliseconds and the intensity of the light the same. Step in bottom trace shows when the light went on. Time pips are spaced at 0.1-second intervals. Picture 9 is a one millivolt calibration applying to all pictures.

Here the electrode is probably in one of the retinula cells rather than in the eccentric cell. The bumps probably originate in the rhabdomeres and, since the electrode is closer to the rhabdomeres in this experiment than in the preceding experiment, the relative differences in distances is greater in the experiment under discussion than in the preceding experiment. Consequently, there is more relative attenuation here which makes greater variation in bump size.

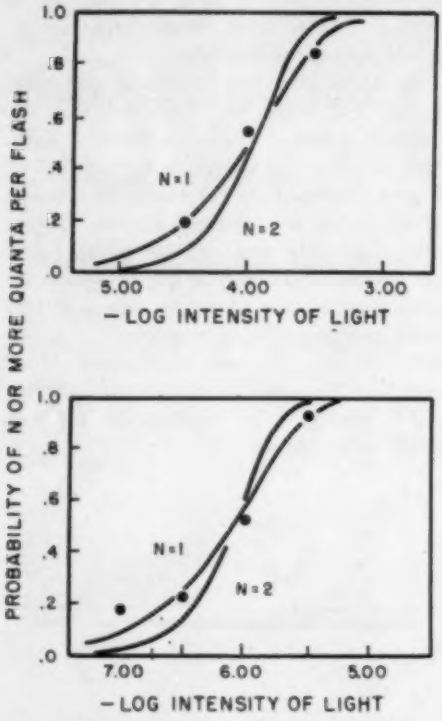

Fig. 11 (Armington and Crampton). Frequency of response as a function of —log intensity of light for two experiments. Response was taken as any deflection in the base line. Points are experimentally determined frequencies. Lines are theoretic curves for one and two quanta. Top graph shows results of first experiment. Bottom graph shows results of second experiment.

TABLE FOR FIRST EXPERIMENT

—Log intensity of light	Fraction of pulses producing a response
4.50	11/34 = 0.32
4.00	17/31 = 0.55
3.50	28/30 = 0.93
blank	2/27 = 0.07

TABLE FOR SECOND EXPERIMENT

7.00	40/51 = 0.79
6.50	25/46 = 0.54
6.00	10/48 = 0.21
5.50	10/56 = 0.18
blank	1/39 = 0.025

In two experiments of the type just described, where the electrode is probably not in the eccentric cell, an attempt was made to determine the minimum number of quanta necessary to cause a bump by the use of Poisson statistics.

It is important to realize that, in determining the minimum number of quanta necessary to cause a bump, variation in the size of bumps makes no difference in the use of this statistical method. It is only necessary that the experimenter be able to tell if a bump did or did not occur after a pulse of light. Despite the variability in bump size it was always possible to tell this. Therefore it is valid to use this method in this case.

In the caption for Figure 11 are shown the results of the two experiments. Each table represents one experiment. In these experiments a 30 millisecond pulse of light was presented to the eye once every 10 seconds. The intensity of the light was varied from pulse to pulse. The denominator of the fractions in the table are the number of trials at the corresponding intensity indicated in the table while the numerator is the number of pulses at the given intensity which gave a response. In the first experiment the intensities of the pulses were not randomized while in the second they were. Any deflection in the base line which occurred within one second after the light pulse was counted as a response. To check against spontaneous activity a blank run was made with the light turned off, and the presence or absence of a response noted during one second intervals spaced every 10 seconds. In the second experiment the blanks were randomly interspersed among the pulses of light.

Figure 11 shows the graphs of frequency of seeing curves for the two experiments.

In both experiments the data fit the theoretic curve for one or more quanta required for a response.

It is tempting to make the hypothesis from these data that only one quantum absorbed can cause a response. However, more experiments are necessary to confirm this hypothesis.

FUORTES: Thank you very much. Dr. Yeandle. It is now intermission time.

PHYSICAL FACTORS IN THE CORRELATION OF ELECTRORETINOGRAM SPECTRAL SENSITIVITY CURVES WITH VISUAL PIGMENTS*

EBERHARD DODT, M.D.
Bethesda, Maryland

The spectral sensitivity of the eye reflects not only the absorption spectrum of visual pigments but also the spectral characteristics of pre-and postretinal structures which, by either absorption or reflection, can change the amount of light falling on the photoreceptor layer of the retina. This report concerns experiments which were carried out to determine how much the absorption of the crystalline lens and the reflectivity of the fundus contribute to the shape of the spectral sensitivity curve of the eye as measured by the electroretinogram.

It is generally assumed that the human lens acts as a color filter reducing the retinal sensitivity in the short wavelength region of the spectrum. A collection of data on lens absorption in both human and animal subjects is shown in Figure 1. In order to study lens absorption by means of the electroretinogram, an Xenon high-pressure lamp with double interference filters was used for stimulation. The filters were carefully selected to insure both sufficient intensity as well as monochromism. A series of experiments were performed on two aphakic human subjects, aged 26 and 37 years, and on two normal subjects, aged 26 and 61 years.

The relative spectral sensitivity obtained in the visible part of the spectrum from both normal and aphakic eyes shows a rather close correspondence to Wald's psychophysical curve for rod vision, Figure 2. A similar correspondence is obtained in the ultraviolet region for the aphakic eyes (filled circles). In the case of the young, normal subject the ultraviolet sensitivity lies above Wald's curve (open circles) and is even more elevated in the old, normal eye (crossed circles). Since the absorption of the lens for short wavelengths is known to in-

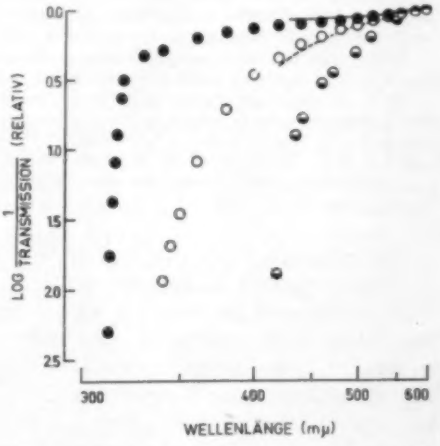

Fig. 1 (Dodt). Relative absorption (density units) of crystalline lens. Spectrophotometric measurements. Young cat—filled circles, old cat—open circles. Continuous line represents Weale's (1954 a) determination of three cat lenses. Human lenses, 48 and 53 years old (Weale, 1954 b), are shown by broken line; 68-year-old human lens (Wald, 1949) given by half-filled circles. From Dodt, E., and Walther, J. B.: Netzhautsensitivitaet,, Linsenabsorption und physikalische Lichtstreuung. Pflüger's Arch., **266**:167-174, 1958.

* From the William G. Kerckhoff-Herzforschungsinstitut der Max-Planck-Gesellschaft, Bad Nauheim, Germany. The work described was supported by grants from the Deutsche Forschungsgemeinschaft toward the cost of apparatus.

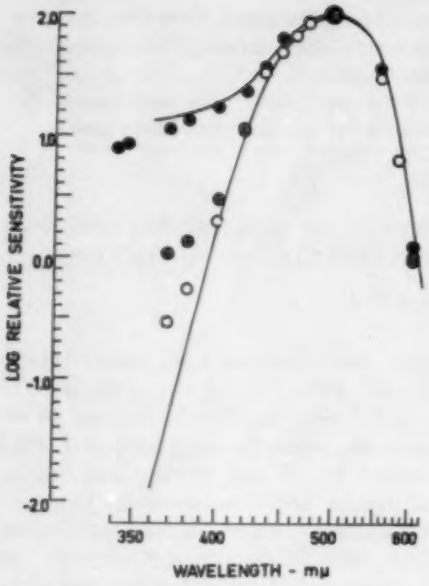

Fig. 2 (Dodt). Average spectral sensitivity of two aphakic eyes—filled circles, one normal eye, age 26—open circles, and one normal eye, age 61—crossed circles as determined by measurement of energies producing scotopic b-waves of constant size (200 μV). Spectrum of equal quantum intensity. Curves drawn in full represent spectral sensitivity of rod vision in an area eight degrees above the fovea in the normal and aphakic eye, as determined psychophysically by Wald (1949). From Dodt, E., and Walther, J. B.: Fluorescence of the crystalline lens and electroretinographic sensitivity determinations. Nature, **181**:286-287, 1958.

crease with age, lower sensitivities would have been expected in the older eye.

If one observes the lens of a normal eye illuminated with ultraviolet light strong enough to cause a medium-sized electroretinographic response, it appears as a whitish patch in a dark surround, that is, it fluoresces. The upper limit of the spectral region, where fluorescence occurs, lies at a relatively longer wavelength in the aged than in young people. It is tempting, therefore, to correlate this with the observed increase of spectral sensitivity which also appears to depend on age. In regard to the electroretinogram, the effects of lens fluorescence are of two types, both of which tend to increase the spectral sensitivity. In the first case, the ultraviolet light absorbed by the lens is reemitted at longer wavelengths for which the retina is particularly sensitive. Secondly, a fluorescent lens acts as a new light source thereby increasing the area illuminated. As has been recently shown by Crampton and Armington, the size of the field seems to be a very important factor in determining the size of the b-wave of the electroretinogram in man.

Since an increase in ultraviolet sensitivity is shown only by the electroretinogram in the present case, the wavelength effect of fluorescence appears to be of less importance as compared to the effect of increased area. In order to test this hypothesis the following experiments were performed on dark-adapted pigmented rabbits. After the eye had been opened and the lens removed, the spectral sensitivity was measured. Then sodium fluorescein was injected intravenously

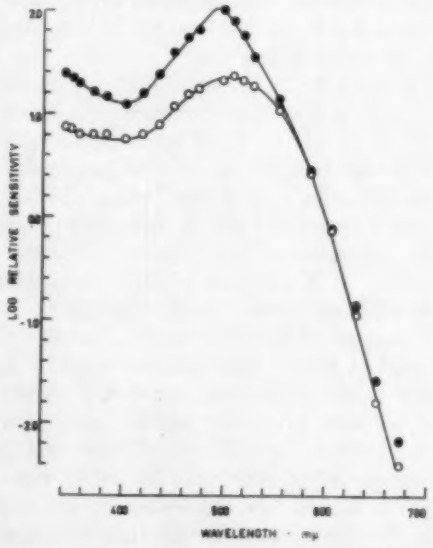

Fig. 3 (Dodt). Relative spectral sensitivity of pigmented rabbit. Lensless, dark-adapted eye. Open circles before, filled circles after injection of sodium fluorescein (2.0 by 50 mg./kg., intravenously). Small area illumination. Equal quantum intensity spectrum. From Dodt, E., Engelkind, R., Quaranta, C., Walther, J. B., and Wirth, A., in preparation.

and the sensitivity measurement repeated. If only a small area was illuminated, the spectral sensitivity below 560 mμ was found to be much higher after fluorescein (fig. 3). Since the absorption maximum of sodium fluorescein at pH 7.2 is located at about the same wavelengths as the maximum of the visibility curve of the rabbit, the increase in sensitivity after fluorescein has to be attributed to the increase in area due to the stray light effect of fluorescence by the preretinal media. Additional proof for this conclusion is provided by the fact that the spectral sensitivity curve after sodium fluorescein did not change if the retina was stimulated by a large field.

In conclusion, I would like to speak about recent experiments which were done in order to elucidate the effect of the pigment epithelium on the shape of the electroretinogram spectral sensitivity curve. In albino rabbits and rats, Granit, Wirth, and others found a narrow sensitivity distribution in the blue-green and a hump in the red part of the visible spectrum. We recently extended these measurements to the lensless eyes of both albino and pigmented rabbits. On the extreme violet and ultraviolet the thresholds for both pigmented and albino animals were found to be approximately the same. For the rest of the spectrum, however, the sensitivity differed very much. In pigmented rabbits it closely resembled the absorption spectrum of rhodopsin, whereas in albino rabbits it was similar to the curve measured by Wirth (fig. 4). The differences in spectral sensitivity between pigmented and albino rabbits were found to be maximal in the blue-green and red part of the spectrum. Similar results were obtained when pigmented and albino guinea pigs were studied.

Since the retinal fundus of an albino is characterized by the absence of pigment in the pigment epithelium, light entering this eye is diffusely reflected from the post-

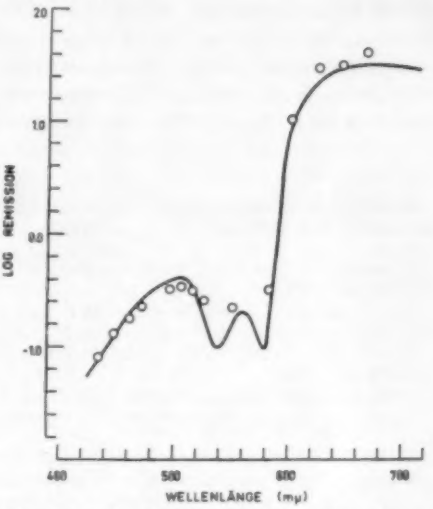

Fig. 5 (Dodt). Spectral reflectivity of blood (continuous line) as compared with the differences in spectral sensitivity between pigmented and albino rabbits—open circles, taken from Figure 4 and enlarged by 1.75 to fit the spectro-reflectrometric data. Reprinted, by permission, from Dodt, E., and Walther, J. B.: Spektrale Sensitivitaet und Pigmentepithel. Vergleichende Untersuchungen an pigmentierten und albinotischen Netzhaeuten. Pflüger's Arch., **266**:187-192, 1958.

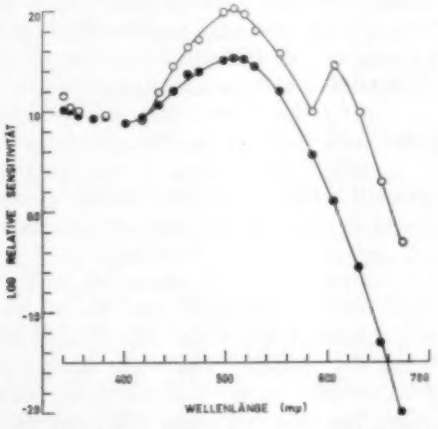

Fig. 4 (Dodt). Relative spectral sensitivity of dark-adapted rabbits. Lensless eyes. Open circles, albino rabbits. Filled circles, pigmented rabbits. Equal quantum intensity spectrum. From Dodt, E., and Walther, J. B.: Spektrale Sensitivitaet und Pigmentepithel. Vergleichende Untersuchungen an pigmentierten und albinotischen Netzhaeuten, Pflüger's Arch., **266**:187-192, 1958.

retinal tissue containing blood. When the difference in spectral sensitivity between albino and pigmented rabbits is compared with the reflectivity of blood, a close correspondence is found (fig. 5). We may therefore conclude that in the albino eye the lack of pigment in the pigment epithelium, and not photosensitive substances, is responsible for its increased sensitivity in the blue-green and in the red.

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Discussion

FUORTES: It is very nice to see that we are now ahead of time instead of being behind time as we have been throughout this morning, and I wonder if there are questions or comments.

ARMINGTON: When the spectral sensitivity of the electroretinogram is measured in the scotopic eye, maximum sensitivity is often found for wavelengths shorter than those (around 500 mμ) giving maximum sensitivity for psychophysic determinations. Dr. Dodt suggested that this shift may be due to fluorescence. To check this we borrowed from Dr. Dodt a filter which transmits very nearly uniformly to almost 400 mμ and cuts down very sharply thereafter. This filter should remove any ultraviolet which may cause fluorescence. Actually, our stimulating light never had much ultraviolet in it because we use glass optics and a tungsten source operated at a fairly low temperature. Anyhow, we found that the same curve with the same unusually high sensitivity in the blue was obtained no matter whether or not the ultraviolet filter was used. Thus, the discrepancy cannot be explained by fluorescence alone. It has been proposed by several investigators that Rayleigh scatter may be important but correction for this effect does not eliminate all difficulties. One may think of contributions from a blue receptor process, but, if that is the case, this process seems to be resistant to chromatic adaptations, unlike other parts of the spectral curve. We think that reflection in the eye should also be considered but still we cannot fully account for this discrepancy.

FUORTES: Thank you very much, John.

RUSHTON: I am rather glad that the hemoglobin effect was mentioned by Dr. Dodt. I had also worked it out in the same way from Wirth's paper, but there were two things that upset me about that interpretation. With a large field from the back of the eye, I should think that the contribution could not be more than equal to the light directly incident on the retina. But one would require the contribution by reflection to be much

more in order to account for Wirth's results. The second trouble was, that the hemoglobin absorbs very highly indeed in the blue violet region and it's in the blue violet that Dr. Dodt has found no discrepancy at all. If the absorption bands in the green are contributing as much as Dr. Dodt finds, I would have thought the Soret band, I think it's called, in the blue violet would contribute a very great deal more. I wonder whether it is possible that Wirth did not direct his light through the dilated pupil but let the light fall indiscriminately on the whole eye so that in the albino rabbit the pink iris was acting as an interposed filter.

FUORTES: Thank you very much. Dr. Dodt is ready to reply but there may be more questions from the floor. Would anybody else like to comment?

WALD: I want to ask Dr. Rushton a question. How does it help to use the iris as a red filter? I see that it helps you explain why reflectance cannot do more than double the effective brightness, but that Soret band of hemoglobins will still be working in the iris, won't it? Or have I missed something?

RUSHTON: No. The Soret band remains as an outstanding trouble with me.

WALD: I am satisfied.

FUORTES: Thank you to both Dr. Wald and Dr. Rushton. May we hear Dr. Dodt now?

DODT: Concerning the blue discrepancy mentioned by Dr. Armington I think very much depends on the curve used for comparison. If, for instance, one compares electroretinogram sensitivity curve with the C.I.E. curve one gets certain discrepancies and, if one compares it with Wald's curve of rod sensitivity one obtains different discrepancies. In the present experiments we located the maximum somewhere between 497 and 507 mμ; the threshold for 473 mμ was definitely higher.

The sensitivity measurements on albino rabbits are based on data from open eyes of which the iris was cut. In order to prevent the light from passing the sclera a focused beam was used. As pointed out by Dr. Rushton, reflection alone cannot account for the increase in spectral sensitivity in the albino eye. Our stimulus beam was rather narrow and thus only a small retinal spot was stimulated directly. By reflection from this spot, however, light is scattered over a much larger area. One may assume that the relation between area and b-wave mentioned above may account for the exaggerated effect of reflectivity of blood on the electroretinogram in the albino rabbit.

INTRARETINAL RECORDING IN THE UNOPENED CAT EYE*

KENNETH T. BROWN, PH.D., AND TORSTEN N. WIESEL, M.D.
Baltimore, Maryland

This communication will give a summary of our work during the last two years. Research has progressed through four stages and these stages will be presented in chronological sequence, as follows: (1) Development of methods for using micropipette electrodes in the unopened cat eye, (2) determination of the sequence of events during a retinal penetration and the development of physiologic methods for localizing the electrode in the retina, (3) study of localization of the different components of the electroretinogram, (4) study of discharge patterns from ganglion cells and cells of the inner nuclear layer.

* From the Wilmer Institute of The Johns Hopkins University and Hospital.

METHODS

Methods for using the unopened cat eye

were essentially the same as those described by Talbot and Kuffler.[3] A two-beam version of the multibeam ophthalmoscope was employed. A tungsten bulb was used in one beam for background illumination of the retina, while a glow tube was the light source in the stimulus beam. A viewing beam was also provided. Stimulus spots were accurately focused on the retina, and all relevant parameters of the stimulus spot were controlled. Micropipette electrodes with tip diameters less than 0.5 μ were filled with 3M-KCl, and D.C. resistance of the electrodes was 10-30 MΩ. These electrodes were inserted into the fundus just behind the limbus, using the method described by Talbot and Kuffler.[3] Thus the active electrode approached the retina from the vitreal side. The micropipette electrode could be aimed at any part of the nasal retina or the central portion of the temporal retina. A remotely operated hydraulic drive was used to advance the electrode along its own axis. The indifferent electrode was always on the back of the head, and standard amplifying and recording equipment was employed.

Movements of the retina relative to the electrode are much more critical with micropipette electrodes than with wire surface electrodes. The animal was maintained under light barbiturate anesthesia, striated muscle movements were prevented by continuous intravenous infusion of succinylcholine, and the animal was artificially respirated. Respiratory movements of the retina were virtually eliminated by unilateral pneumothorax. The intraocular pressure of the closed eye is also very important for minimizing respiratory and pulse movements of the retina. These techniques for controlling movement have proved adequate for present purposes. The preparation can usually be maintained in excellent condition for over 24 hours.

Results

1. The sequence of events during a retinal penetration

The following description of a retinal penetration will summarize our experience from several thousand penetrations. It should first be pointed out that a micropipette electrode is a very sensitive transducer, and deformations of the electrode tip by pulse movements are recorded faithfully in the voltage record. When the electrode is in the vitreous humor, the recording baseline is steady. As soon as the retina is contacted, the pulse beat of the surface vessels appears in the record. Thus the pulse beat provides a sensitive index of when the retina is contacted and gives the zero point from which all retinal depths are measured. The surface blood vessels of the retina occupy the zone from the inner limiting membrane to the outer margin of the inner nuclear layer. Beyond the inner nuclear layer there are no deeper blood vessels until the choriocapillaris on the outer surface of Bruch's membrane. In accordance with the histology, a clear pulse beat is recorded by the electrode to about 40 percent of the total retinal depth. Then there is a sudden reduction or disappearence of the pulse beat, which indicates the outer margin of the inner nuclear layer. The pulse beat stays small or absent for the remainder of the retinal depth and then suddenly returns. The return of the pulse beat indicates contact with Bruch's membrane. If the membrane is penetrated, the pulse beat remains during further advance of the electrode through the vascular choroid. Thus pulse recording allows positive identification of three cardinal landmarks during a retinal penetration. The retinal surface, outer margin of the inner nuclear layer, and Bruch's membrane can be identified on every penetration.

Just after penetrating the inner limiting membrane, impulse activity is frequently recorded from single units of the ganglionic layer. Slightly deeper a second layer of single unit impulse activity is encountered in the inner nuclear layer. Just after leaving the inner nuclear layer, single units which do not discharge impulses but respond to light with slow potentials are sometimes detected. The external limiting membrane is difficult to detect, but occasionally a negative

transient occurs about 30 to 50μ prior to Bruch's membrane, presumably as the external limiting membrane is passed. This result corresponds to what one might expect since the external limiting membrane, as described by Sjøstrand,[2] is not a continuous membrane. When Bruch's membrane is penetrated, a 30 to 60 mV negative D.C. shift occurs and the entire electroretinogram is sharply reduced in magnitude.

Two points should now be made before proceeding:

First, the correlation between electrical events during a retinal penetration and retinal histology is so exact that the electrical events can be used to identify specific anatomic structures. Thus methods are available for localizing the electrode within the retina without relying upon measured depth from the retinal surface.

Second, Brindley[3] described in the frog retina a specific membrane with a high electrical resistance. This membrane supported a 10 to 30 mV potential, with the choroidal side negative, and most of the electroretinogram was recorded across the electrical resistance of the membrane. Brindley designated this as the "R membrane" and tentatively identified it as the external limiting membrane. It is apparent that there is a membrane of similar properties in the cat. In this work the external limiting membrane and Bruch's membrane could be identified separately, and the "R membrane" of the cat was clearly identified as Bruch's membrane.

2. Localization of electroretinogram components

When the active electrode is in the vitreous humor, the electroretinogram appears the same as with a corneal electrode. In addition to the well known a-, b-, and c-waves, there is a positive D.C. component. All four of these components reverse polarity as the retina is penetrated, which leads to the comforting conclusion that all four components are generated within the retina.

The D.C. component may be isolated by reducing the stimulus intensity. When the electrode is in the vitreous humor, the a-, c-, and b-waves disappear in sequence as the stimulus intensity is reduced, leaving only the positive D.C. component. If the electrode is between the external limiting membrane and Bruch's membrane, the three well known waves drop out in the same sequence as stimulus intensity is reduced, leaving only a negative D.C. component. Thus the cat electroretinogram can be analyzed clearly into four major components.

Four kinds of evidence have been obtained concerning the retinal sites at which the different components of the electroretinogram are generated. These types of evidence are as follows: (1) localization of the point at which a maximal response amplitude is obtained, (2) localization of the point at which a polarity reversal occurs, (3) differential effects of local anesthetic upon the different electroretinogram components, (4) intracellular recording.

Localization of the point at which a maximal response amplitude occurs has proved possible for all four components, and the interpretation of these results seems straight forward. The a-wave disappears as the retina is penetrated and then reappears with an inverted, positive polarity in the region of the outer nuclear layer. The amplitude of the positive a-wave then increases and reaches a maximum just before Bruch's membrane is passed. The amplitude drops sharply as the membrane is penetrated. The c-wave has a positive polarity at all levels prior to Bruch's membrane. Its amplitude also increases to a maximum just before passing Bruch's membrane, and when the membrane is penetrated the polarity reverses and the response amplitude drops. The b-wave and D.C. component have amplitude-depth curves which are essentially identical. These components are both recorded with negative, inverted polarity after the outer margin of the inner nuclear layer has been passed. The amplitude then increases quickly to a maximum, which seems to be in the external plexiform layer or the

inner margin of the outer nuclear layer. Subsequently the amplitude falls to a plateau, which is maintained until Bruch's membrane is penetrated.

The only electroretinogram component with a reversal point which can be localized precisely is the c-wave, which reverses polarity as Bruch's membrane is penetrated. Technical problems have made the other reversal points difficult to establish, and on theoretic grounds the reversal points of the other components seem less valuable than amplitude maxima for localizing the components.

Local anesthetics which block impulse transmission should block any electroretinogram components which depend upon transmitted impulses. It has been found that Xylocaine, when injected in small doses into the vitreous humor near the recording electrode, will produce a complete and reversible block of the b-wave. The other components are not affected by Xylocaine. These findings indicate that the b-wave is dependent upon transmitted impulses, while the other components are not.

In a few cases intracellular recordings have been obtained from single units immediately adjacent to the vitreal surface of Bruch's membrane. It seems very unlikely that intracellular records were obtained from outer segments of receptors, because of the extremely small size of these structures, so the recordings were most likely from single cells of the pigment epithelium. The intracellular recordings have been unstable, as would be expected with such small cells, but membrane potentials of 30 to 60 mV have been recorded. The light responses in these intracellular recordings show a slow potential with the same time course as the c-wave, but the intracellular potential is inverted in polarity with respect to the extracellular c-wave and much larger in size. Thus it seems likely that the c-wave has been recorded intracellularly from single cells of the pigment epithelium.

Certain other intracellular responses are comparable to those reported in fish retinas by MacNichol and Svaetichin.[4] Let us consider these intracellular recordings first in relation to similar recordings from fish, and then in relation to the b-wave and D.C. component of the electroretinogram.

No impulse activity is found from such units; only slow potentials are produced by light stimuli. The form of the potential is quite similar to certain responses obtained from fish retinas, and the response is well maintained with a long light stimulus. The response to white light is always a hyperpolarization and its magnitude is graded with stimulus intensity. There are, however, interesting differences. Whereas intracellular recordings of this kind are easily made in fish, they are extremely difficult to obtain in the cat retina. Only two stable intracellular recordings of this kind have been obtained, although a number of fragmentary observations have been made. Thus the single units which yield these responses are probably much smaller in the cat than in the fish. In the cat these responses seem to be localized slightly external to the outer margin of the inner nuclear layer, that is, in the region of the external plexiform layer.

Although this localization may differ slightly from the localization of the luminosity response by MacNichol and Svaetichin,[4] the difference is very small in terms of the accuracy of localizing techniques and histologic differences between cat and fish retinas. A rather large number of extracellular single unit recordings have been obtained from apparently the same cells, and the polarity of the response has thus far been the same with intra- and extracellular recording from single units. This point is not understood.

In relating these intracellular responses to the b-wave and D.C. component, it seems clear that the single unit responses are obtained from about the same retinal location where the maximal amplitude is found for the b-wave and D.C. component. The form of the intracellular response is very similar to recordings of the b-wave and D.C. com-

ponent. Also with short stimuli, on the order of 40 msec., it has been observed that the intracellular response breaks into distinct fast and slow components. These findings suggest that intracellular recordings have been obtained from the cells which generate the b-wave and D.C. component.

In summary, all the evidence on localization of the four major components of the cat electroretinogram is internally consistent. The c-wave is probably generated in the pigment epithelium. The a-wave also comes from some structure very close to the vitreal side of Bruch's membrane, probably the receptor outer segments. The b-wave and D.C. component are generated by structures farther back in the retina, in the region of the external plexiform layer.

3. Discharge patterns from single cells

Single units of the inner nuclear layer may be identified by four criteria, as follows:

1. The ganglion cells of the peripheral cat retina constitute a single layer of scattered cells, with two ganglion cells almost never overlying one another. Thus if two cells which discharge impulses are found in sequence on the same penetration, the second cell is almost certainly in the inner nuclear layer.

2. The b-wave does not invert polarity until the region of the inner nuclear layer, so a negative b-wave at the recording site is another criterion.

3. The distance which must be traversed after leaving the cell, in order to pass the outer margin of the inner nuclear layer, should be 20 μ or less.

4. The last and crudest criterion is depth from the retinal surface, which should exceed 40 μ.

Single cells can be found which satisfy all four criteria, and in such cases the identification seems certain. Cells of the inner nuclear layer, identified in this manner, have been found to yield both spontaneous and light induced impulse activity. Receptive fields of cells studied to date in this layer have been organized like those of ganglion cells. In the light-adapted state there is either an "on" center and "off" periphery, or vice versa, and mutual inhibition occurs between central and peripheral portions of the receptive field.

A new type of response pattern has now been found consisting of pure inhibition, with no sign of an "off" discharge. The response is best obtained with a small stimulus spot in the center of the receptive field, and this response type has been found from certain cells of both ganglionic and inner nuclear layers. The typical nature of the response to a 1.0 second stimulus is complete inhibition of the maintained discharge immediately after the light is turned on, followed by a decrease of inhibition to a plateau while the light is still on. When the light is turned off, complete inhibition occurs again for as long as several seconds, followed by a slow return of the pre-stimulus discharge frequency.

The inhibitory response is delicately graded with stimulus intensity; a higher intensity produces stronger inhibitory effects. All cells studied to date have shown some degree of "off" discharge in the intermediate range of intensities, but a purely inhibitory response is obtained at high and low intensities.

A few stable intracellular recordings have been obtained from ganglion cells, with membrane potentials on the order of 50 to 60 mV. An observation of particular interest is that an "on" discharge, maintained during a 0.8 second stimulus, is accompanied by a maintained depolarization. In all cases seen to date, inhibition of impulse activity has been associated with hyperpolarization.

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Discussion

Gouras: I think that the observation that increasing intensities of illumination can curtail the frequency of single unit "off" discharges is of great interest. It confirms Hartline's observations on the single optic nerve fibers of amphibians in which he obtained a decreased frequency of unit discharges with intensities six log units above threshold especially in the case of "off" units. I have been particularly impressed by the curtailment of the duration more than the frequency of discharges with increasing intensities in the case of toad retina.

I would like to ask Dr. Brown whether by selectively blocking the b-wave he has measured either changes in the latency or amplitude of the a-wave?

Fuortes: Dr. Forbes.

Forbes: The apparent inhibitory action of the stimulus producing the "off" effect reminds me of some early observations of Sherrington on the inhibition of the extensor reflex. Sherrington showed that stimulation of ipsilateral nerves in the hind leg of a decerebrate cat evokes inhibition of the extensors while the stimulus is on and rebound contraction upon removal of the stimulus. Originally, he interpreted that as the release energy stored during stimulation. He and Miss Sowton later showed that that view was not tenable because when they prolonged the stimulus or increased its intensity they obtained larger inhibitions but smaller rebound contractions. They suggested that the inhibitory stimulus had a suppressed excitatory component which ran concurrently with the inhibition. The inhibition was dominant while the stimulus was applied and, when the stimulus ceased, the excitatory component (having a longer after-effect) appeared. But if they applied enough inhibition, apparently the inhibitory component had itself sufficient after-effect to prevent the excitatory component from making its appearance. Some of Dr. Brown's records on the "off" effect following the stimulus reminded me of that. I noticed that in some cases there was a burst of impulses just after cessation of the stimulus. In other cases, when the stimulus was longer or more intense, the "off" response did not appear and I wonder if that might be explained in the way proposed by Sherrington.

Fuortes: Thank you Dr. Forbes. Dr. Svaetichin.

Svaetichin: I have been disappointed myself in determining the depth of the microelectrode in the retina from readings on the indicator gauge fitted to the micromanipulator. Although we in our preparation can see the exposed cone layer under the dissecting microscope, we found that the gauge gives a very approximate measurement of position of the electrode relative to the retina. Would you explain again the method you use for determining the location of the electrode tip in the retina? My second question is: Do your intra- and extracellular recordings always show the same polarity?

Fuortes: If there are no additional comments, I would like to make a minor comment on Dr. Brown's paper. Is it not rather unlikely to penetrate cell membranes in the external plexiform layer where the structures are so small? And in case it is possible to record intracellularly, where would one expect to pickup the large, steady potentials described? Perhaps Dr. Brown will answer these questions now.

Brown: With regard to the time relation

between the a-and b-waves, as mentioned by Dr. Gouras, we have not measured a-wave latency during blockage of the b-wave by Xylocaine. It is clear that the b-wave begins during the a-wave, and cuts off the recorded amplitude of the a-wave under normal conditions, since blockage of the b-wave produces a great increase in the recorded amplitude of the a-wave.

It seems quite possible that "off" discharges from the cat retina could be produced, at least in certain cases, by a mechanism of the type suggested by Dr. Forbes.

I understand from Dr. Svaetichin that in fish the polarity of the extracellular response is inverted from the intracellular polarity. This inversion of polarity has not been found in the cat, but, since the intracellular polarity is the same in fish and cat, the difference of results seems in the extracellular polarity. We rather often record responses which are similar to the intracellular responses, except that these responses are much smaller in amplitude and are obtained with no sign of a membrane potential. Such responses are highly localized and are found in the same region of the retina as the intracellular responses. We have considered such responses as extracellular recordings from the same single units which yield rare intracellular results. Further work seems required to establish whether the relation between intra- and extracellular responses is really different in fish and cat, and, if so, to establish the basis for the difference.

In reference to Dr. Svaetichin's question about determining electrode depth, we measure electrode depth from the retinal surface but we do not trust this as a method for localizing the electrode. Now that correlations have been established between retinal histology and electrical events during a retinal penetration, the electrical events are used to localize the electrode. This method of localization has made it possible to evaluate depth measurement as a means of electrode localization. The measured depth of a given structure from the retinal surface has proved quite variable, and apparently this finding is in accord with Dr. Svaetichin's experience.

With regard to Dr. Fuortes' question, our evidence indicates that the slow potentials we record, which are comparable to those of MacNichol and Svaetichin, are obtained from very small structures. The single units which give these potentials are very difficult to hold with either intra- or extracellular leads, and recording time is usually only a few minutes. This is in contrast with single units of the inner nuclear layer, which can be held for an hour or more in spite of the fact that the cell diameters are only on the order of four to eight μ. Thus our findings are consistent with the fact that all structures of the external plexiform layer are quite small. Intracellular recording from the external plexiform layer would certainly be a statistically unlikely event. The extreme rarity of intracellular recording bears out that prediction and provides further evidence that the responses are obtained from very small structures. We have entertained the possibility that these slow potentials in the cat retina are recorded from receptor terminals, such as cone pedicles and rod spherules, but that is only speculation. In the present state of knowledge, I do not know whether large, steady, slow potentials should be expected from such structures or not.

FUORTES: Thank you very much, Dr. Brown. Dr. MacNichol.

MACNICHOL: Under some conditions, a reversal of potential does not follow cell penetration. In the case of the Limulus eccentric cell, Dr. Wagner and I have been able to penetrate some of them under visual control. Outside the cell, and in fact anywhere in the ommatidium, one records small positive spikes. Following penetration of the eccentric cell body, the spikes become larger but, of course, remain positive. What we believe this means is that the spike is generated at some distance, perhaps somewhere in the

axon. Concerning electrode depth I think that Dr. Brown is in a better position than Dr. Svaetichin and I were. The reason for this is that he is working in an unopened eye under fluid and in these conditions one may expect less deformation of the retina, following movements of the microelectrode.

FUORTES: Thank you very much, Ted. I wonder if it is worthwhile to point out that Dr. Svaetichin was mentioning the problem of slow waves not reversing from outside to inside, while Dr. MacNichol has discussed the different problem of spikes not reversing from outside to inside. I am not sure that the conditions are the same in the two situations.

There is now another comment from Dr. Svaetichin.

SVAETICHIN: Dr. Brown and Dr. Wiesel presented results which indicate that the c-wave of the electroretinogram originates in the pigment epithelium. Therman showed that the c-wave increased very much when adrenaline was added, and Noell was of the opinion that the c-wave originates in the pigment layer. At the symposium in Caracas, 1957, Porter described the ultrastructure of the myeloid bodies of the pigment cells; it is laminated like the outer limbs of the photoreceptors. In collaboration with Fernández-Morán and Jonasson, we showed (1956) that a large electroretinogram component can be obtained from the cornea of the giant moth Erebus, isolated by microdissection. The isolated cornea preparation contains the crystal lenses and the pigment cells, but no photoreceptors. Thus, a part of the electroretinogram seems to be an "electropigmentogram."

FUORTES: Perhaps Dr. Brown will conclude this discussion.

BROWN: Dr. MacNichol has certainly pointed out one of the reasons why measurements of electrode depth are more accurate in the unopened cat eye than in isolated preparations. I only want to emphasize that the major advantage of the unopened cat eye for electrode localization is that a new method can be used in this preparation which seems inherently much more accurate than depth measurement.

In a slightly more general vein, it would seem that neurophysiology of the mammalian retina has progressed rather slowly by comparison with work on isolated preparations from cold-blooded animals. The emphasis on cold-blooded preparations appears due, at least partly, to feelings that mammals are difficult to work with, and that the complexity of the mamalian retina makes results difficult to interpret. Now that techniques have been developed for using micropipette electrodes in the unopened cat eye, I would like to stress that this preparation can be undertaken with little more difficulty than one encounters with cold-blooded preparations. We have found that the unopened mammalian eye offers many technical advantages and points the way to solution of many fundamental visual problems. It is already quite evident that certain kinds of results are easier to obtain and interpret in the unopened mammalian eye than in cold-blooded preparations. I wish to stress these points because I feel they are important in terms of the over-all progress of visual neurophysiology.

FUORTES: Thank you very much. I think this concludes the afternoon session and I hope that most of us will meet here again tomorrow morning at nine o'clock.

TEMPORAL PERIODICITIES IN THE PRIMARY PROJECTION SYSTEM*

WADE H. MARSHALL
Bethesda, Maryland

The problems of vision present a great number of facets and attract many kinds of specialists with different ideas and inquiries. Cajal and his students, immersed in meticulous study of anatomic detail, were certainly respectful of the complexities of neuron interconnections and not aware of the physiologic possibilities of unchanneled, or avalanche conduction. While the study of anatomic detail indicated synaptic discontinuities to some observers, others made the interpretation that there were structural connections between neurons which would theoretically permit activity to progress throughout the nervous system in an unchanneled way.

Many of us came on the scene near the beginning of the electronic age at the time of great developments in axonology. It has been only recently in reading Polyak's recent and final work that I have appreciated the extent of the arguments between the strict constructionists of the neuron doctrine and the reticularists. The former believed, if one can make an over-simplified statement, that a neuron consisted of a dendrite to conduct coded information to a cell, and a cell to integrate the pulses and discharge the axon to the next station. The dendrite might be specialized, in fact it was usually considered to be highly specific, with different types of cells and axons for different functions. Each unit operated as a cog in the machine, the proper operation of which depended on complex interaction of all-or-none units.

The syncitial system, on the other hand, was not so limited by anatomic organization but was governed by various kinds of mystical field forces and dynamic processes.

It is now generally recognized that there is no place for extreme views on either side of this argument. The facts of axonology and all-or-none digital type computation must be integrated into the graded response mechanisms of the short soma appendages. For a discussion of this general problem I refer the reader to Dr. George Bishop's *Natural History of Nerve Impulse*.

It is now very doubtful that there are any simple neuron systems in which activity in one neuron, for instance, is not influenced by activity in contiguous neurons. Various kinds of interaction occur which are not simply related to active transmission across synapses. This can be demonstrated even in myelinated fibers of a nerve trunk if electric shock stimulation is used to fire a group in synchrony. It is doubtful that such interaction is of physiologic importance (Gasser, 1955) but it is a good example for the extreme case.

That similar types of electric (and chemical) field interaction occur in the central nervous system ganglia including retina and cortex there is no doubt, but the precise physiologic role of such forces is difficult to quantitate. What is unquestionably important is that demonstrable synaptic mechanisms operating on reciprocally overlapping graded response dendrites do provide interacting mechanisms which bear some resemblance to syncitial mechanisms. Since electric studies of the central nervous system beginning with the electronic age were an outgrowth of axonology, the digital aspects of operation were, perhaps, overemphasized.

It is to be noted that there is one clear example of avalanche conduction: the spreading cortical depression of Leão. This phenomenon occurs in cortex which has been tampered with for experimental purposes. This is a slow moving reaction involving the entire cortical matrix and seems to propa-

* From the Laboratory of Neurophysiology, National Institute of Metal Health, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

gate with no regard for circuitry or even synaptic mechanisms and under favorable conditions may repeat in apparently spontaneous cycles for hours. A similar phenomenon which occurs in amphibian retinal preparations has been specifically examined by Gouras (1958). This reaction appears to serve no physiologic purpose except to confuse experimental work. But it does illustrate that the cortical and retinal networks are vulnerable to avalanche type reactions under pathologic conditions.

The retina, the lateral geniculate, and the striate cortex consist of elaborate ganglia connected by axons which can carry information only by frequency modulation. Spatial organization is but one aspect of visual integration. Related to spatial pattern is modulation in time which is a universal characteristic of the central nervous system.

At the cortical end of the system we encounter the alpha process which for the cat is about five per second and for man is about 10 per second. This periodicity has a definite relation to subjective sensation as was shown by Bartley (1938a, 1938b, 1939, 1941). At the input end of the visual system we are confronted with a more or less continuously moving image. In recent years very ingenious experiments have been done on the retina (Riggs, et al., 1953; Ditchburn, et al., 1955a, 1955b, 1956; and Krauskopf and Kalla, 1956).

It is generally agreed that pattern vision soon fades and disappears within one to five seconds. If the source is interrupted at less than fusion frequency pattern vision is restored. The normal small movements of the eye (physiologic nystagmus) have been shown to enhance contrast threshold and acuity as tested by percent time seen using various simple patterns.

Conversely controlled movement of the image on the retina has shown that periods of one, two, and five cps result in lowered thresholds, and that higher frequencies of image motion result in higher thresholds for bars subtending four minutes of angle or less. However, the Krauskopf and Kalla experiments show that for bars of eight minutes of angle frequencies of 10, 20, and 50 cps result in decreased threshold. These investigators express doubt of the validity of these data for the eight-minute bar.

In any case the fact of the importance of eye movements for sustained visual acuity is established. The case for the higher frequency components is still in question.

Consideration of the time intensity function of the retinal process indicates that there must be an upper limit to velocity of image transients above which stimulation assignable to movement per se is inconsequential at equilibrated levels of photochemical adjustment of brightness. At the equilibrated level quick shifts (flicks) probably produce inconsequential stimulation because the velocity of image movement is too high. However, Ditchburn believes that he has evidence for an active inhibitory process during the flick (1956). This has also been suggested by Lindsley (1952).

There are two aspects of the image movement problem. For slow movements the rate of change of stimulus may be considered in terms of a derivative as Ditchburn has suggested (1956). There is also time for action of lateral and vertical interaction processes as the image moves (Marshall and Talbot, 1942). For displacements occurring above some as yet unestablished velocity little effect of the movement per se can be expected. The spatial displacement is effective, however, in producing an abrupt change in stimulus pattern, which may result in consequences not significantly different from movement at finite velocity.

The question of movement per se versus space displacement will probably be considerably resolved by current work of Hartline and his collaborators. Riggs, et al. (1956), using the stabilized image and variable presentation intervals of test pattern, found that for short intervals threshold for the stabilized image is higher. Their data indicates that somewhere between 0.1 and 5.0 seconds,

eye movements aid acuity. This is an important experiment but the answer does not exclude the possibility that image displacements over the shorter time intervals play a positive role in normal visual activity.

There is much suggestive evidence that the temporal discontinuities in the neural processes are crucially involved in these time ranges 0.01 to 1.0 second. Adrian, impressed by the discharges recorded from the optic nerve following change of stimulus, considered the general proposition that eye movements may be constructively involved in the visual process (Adrian, 1928). Later it was observed that the thalamic relay nuclei exhibited the capacity of transmitting information in groups of three to five pulses at high frequency under nembutal anesthesia, after which the synaptic transfer mechanism exhibits subnormality for an interval of the order of one second and is absolutely refractory for a period of about 30 to 50 msec. (Marshall, 1941, 1949).

The thalamic relay nucleus thus tends to make transmission discontinous, and further aid in accentuating brief trains of transmission following a change in stimulus intensity at peripheral end-organs. The over-all recovery time of optic nerve to cortex was found to be over one second. The data then available about physiologic nystagmus indicated three distributions of eye movements roughly grouped in a 10 to 100 per second range, a five per second range, and a one-second range. It was speculated that these periods and amplitudes yielded velocities of transit across the retinal mosaic which result in discontinous stimulation of receptors, at intervals important in the recovery cycle. It was pointed out that this essential discontinuity of stimulation is necessary for continous vision in a fatigable system (Marshall and Talbot, 1942).

The stabilized image experiments already discussed have substantially confirmed this inference except for the higher range of the three groups of eye movements (which latter is still in question). Whether the fast adaptive effect involved here should be regarded as essentially photochemical or neural is still debatable, with the former favored by most investigators doing the perceptual observations. However, the phenomenon may be similar to many other types of end-organ action regarding response to change of stimulus. The slower photochemical effect may operate mainly for general changes of level of illumination. The central ascending systems operate to favor discontinuous transmission and elaboration of the retinal output into a more temporally organized pattern in the cortical levels.

The experimental examination of time patterns is not simple. There is always to be considered association neurons and partially shifted overlap (Lorente de No, 1934, and Marshall and Talbot, 1942) which result in lateral interaction. Hence spatial and temporal categories cannot be separated. The interaction is both facilitory and inhibitory. Hartline is currently engaged in fundamental analysis of these factors in the retina of the Limulus (Hartline and Ratliff, 1957).*

Much of the data in past years have been taken from anesthetized animals. More recently techniques of implanting electrodes in chronic animals have made it possible to obtain reliable comparisons of unanesthetized and anesthetized preparations.

There are various ways we can examine central transmission. One of the simplest is to stimulate the optic nerve or tract of the cat with electric shocks and record the discharge of the radiation neuron. It is found that the synapse transfer performs adequately for about 10 msec. In this interval four to five nearly maximal discharges can occur, then the amplitude declines, though an interval of residual facilitation can be shown to persist for the order of 30 msec.

Evarts has shown that pentobarbital an-

* Many other systematic investigations of temporal pattern processes are under way. One example is Fitzhugh's work done in Kuffler's laboratory (Richard Fitzhugh, "The statistical detection of threshold signals in the retina." J. Gen. Physiol., **40**:925-947, 1957).

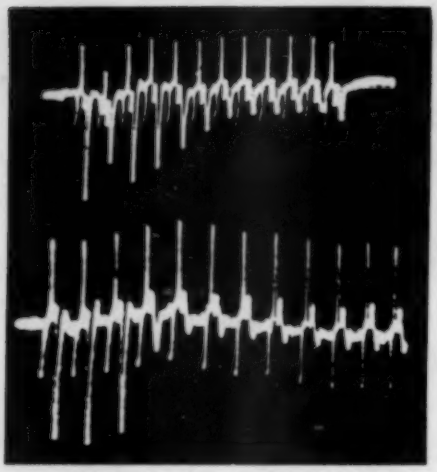
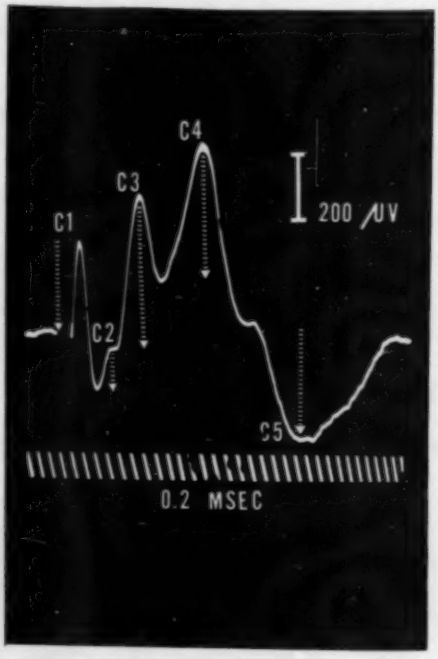


Fig. 1 (Marshall). Records from lateral geniculate nucleus of cat. Contralateral optic nerve stimulated at 400/sec. with electric shocks near maximal for A fibers. The positive spike is tract response, the negative spike is discharge of radiation neuron. The decerebrate preparation was made by intercollicular section and is similar to "cerveau isole" of Bremer. Note longer period of major transmission followed by indications of alternation in the decerebrate preparations. Positive is upward deflection (Evarts, E. V.: unpublished).

esthesia decreases the time during which high frequency discharges take place and makes the ensuing subnormal phase more profound (fig. 1). In either case, the lateral geniculate appears to be able to transmit a significant burst of impulses for a few msec. and then transmission is decreased for an interval of the order of one second. Similar data have been obtained by stimulating the radiation neurons and recording at surface of striate cortex (Schoolman and Evarts, 1957).

Figure 2 is an example of the pattern of a single response following electric shock stimulation of the radiation neurons. Note that the pattern is very similar to the pattern seen under pentobarbital. The only striking difference is that the discontinuity between C4 and C5 is consistently more prominent in the absence of anesthesia.

The activity cycle done with paired shocks under anesthesia is shown in Figure 3 and with no drug in Figure 4. Pentobarbital reduces the response to the test shock during the initial 25 msec. period. Final recovery requires over one second in either case. The anesthetic is thus not responsible for the long total recovery time.



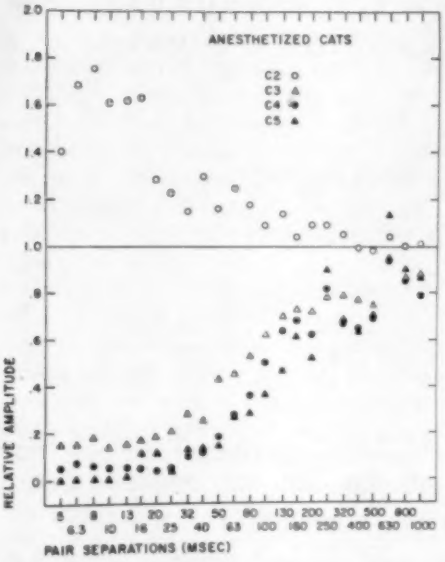


Fig. 3 (Marshall). Curves of recovery cycle obtained by stimulating radiation neurons with paired shocks near maximal for A fibers and recording from surface of striate cortex of cat. Electrodes implanted. Components plotted are labelled as shown in Figure 2. Preparations anesthetized with pentobarbital.

It is interesting to note that George Bishop has maintained for many years that a mixture of ether and $MgSO_4$ produces an anesthetic state that is most nearly physiologic. Schoolman and Evart's recovery cycle data obtained in the conscious cat are very similar to those reported by Clare and Bishop (1952).

Recruitment data taken from geniculate transfer reactions show that pentobarbital shifts the peak of the recruitment curve from the order of 35 msec. to 70 msec. as shown in Figure 5 (Evarts, unpublished). The onset of recruitment is also delayed about 20 msec. If the pattern is elaborated by spinal cord mechanisms, this alteration of recruitment mechanisms at the thalamic level is revealed as an apparent absolutely refractory period (Marshall, 1942; King, Naquet, and Magoun, 1957). If the pattern is elaborated by the retina, that is retina illuminated by paired photic stimuli, a similar effect of pentobarbital is seen (Evarts and Fleming, unpublished). This relation of recruitment to paired electric shocks following a train of electric shocks thus stands in a significant relation to the more physiologic stimulation.

When the cortical system is tested by single shocks to radiations following a photic flash we find a very interesting sequence of events (fig. 6, upper curve). The first peak occurs at about 25 to 30 msec. and is coincident with the surface positive primary response complex to the flash. A second peak occurs at about 130 msec., a third at 230, and a fourth at 430. These periods correspond to the multiple response recorded from striate cortex of the cat reported previously by Marshall, Talbot, and Ades, 1943. This curve

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Fig. 2 (Marshall). Response recorded at surface of striate cortex of cat following single shock stimulus to radiation neurons. Positive is upward deflection. C1 is radiation spike, all subsequent components are considered to be cortical reactions. (From Schoolman, A., and Evarts, E. V.: Study of cortical response to lateral geniculate radiation stimulation in cats with implanted electrodes. J. Neurophysiol., **20**: in press, 1958.

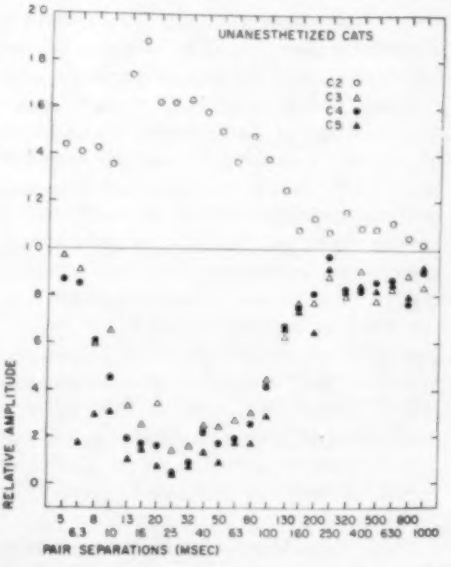

Fig. 4 (Marshall). Same as Figure 3 but with no anethesia; same preparations as used for curves of Figure 3.

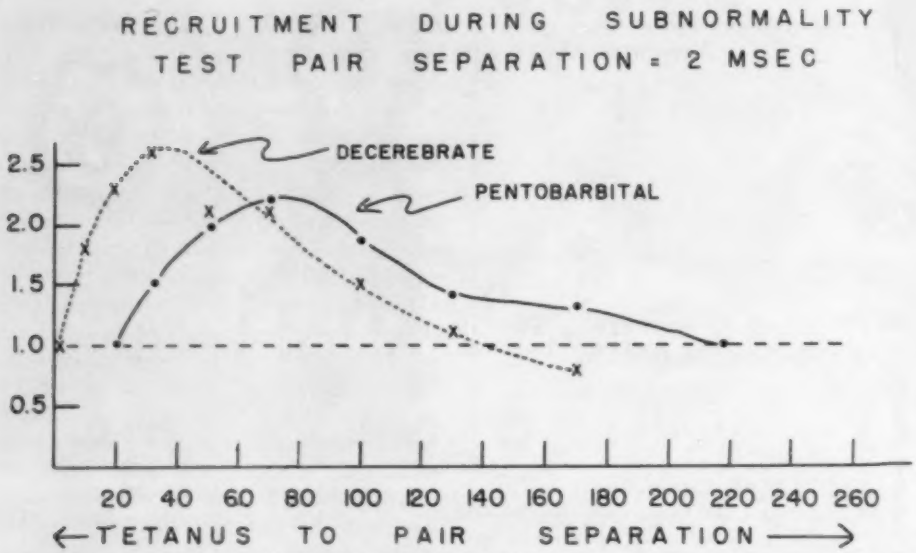

Fig. 5 (Marshall). Curves showing relation of pentobarbital anesthesia to transmission through lateral geniculate of cat. Stimuli applied to contralateral optic nerve, and consist of a conditioning train of 12 shocks at 400/sec. followed by a test pair. Time on X-axis is time from end of tetanus to first of pair. Interval between pair was two msec. for curves of this figure. Values on Y-axis are ratios of second postsynaptic response to first of the pair. Decerebrate preparation was used for unanesthetized case. Implanted electrodes in chronic preparations show similar curves but data are less clear because of interference of ipsilateral reaction.

substantially confirms Chang (1952) who did a similar experiment. The question of the role of the anesthetic here is crucial.

Evarts and Fleming have recently found that the stage of pentobarbital anesthesia is important for evoking the multiple response as tangible electric waves, a fact that was previously overlooked. A periodicity of a similar order of temporal magnitude has been found in the conscious cat in which type of preparation the multiple response is not apparent in cortical records, but this examination of excitability cycle pattern reveals that the fundamental process of periodicity is operating, and that it is not seriously changed by pentobarbital (fig. 6, lower curve). The latency of the first photic response at cortex is prolonged, however, by the drug.

These experiments do much to establish the basis for profitable comparison of these time relations to the alpha process. The alpha process for the cat is about five per second. For many years Bishop, Bartley, and O'Leary have emphasized the alpha process periodicity (Bartley, 1938a, 1938b, 1939, 1941; and Bishop and Clare, 1952; and Clare and Bishop, 1952). The multiple response of Marshall, Talbot, and Ades has been difficult to fit into the alpha process pattern because it is about one half the period of the latter. Evarts and Fleming have shown that under certain conditions the undrugged cat reveals an alpha pattern multiple response. Previous data suggests that the 0.1 second period is driven by the retina though this has not been confirmed recently. The 0.2 second (alpha) period is essentially cortical in origin.

These data show that there are three fundamental time intervals in the primary geniculo-striate projection system of the cat, of approximately 10 msec., 25 msec., and 1.0 second. There are good reasons for believing

that these intervals are due to residual properties of the neurons and are not dependent on circuit activity, though circuit activity (internuncial activity) does, of course, enter into the picture, more so in the absence of anesthetic. The 0.1- and 0.2-second periods are not so simply assignable to the residual properties of the neurons involved.

These characteristic effects of pentobarbital are seen in very low doses (5.0 mg./K), much below the anesthetic level. The chief effect of the anesthetic appears to decrease the effective transmission interval in the neuron systems we have studied. This suggests that mechanisms requiring many sequential links are deranged, not by specific block at any one station, but by a gradual failure due to temporally decreased transmission at each station. As a result the complex reactions necessary for maintenance of the conscious state are not developed.

This general conception has been advanced by many others. French, Verzeano, and Magoun (1953) have suggested that this effect in the multisynaptic brain stem systems is responsible for the apparent differential anesthetic sensitivity of brain stem versus the oligosynaptic primary sensory projection reactions. These data suggest that this difference is one of degree and is merely less evident in the oligosynaptic systems and that the same principle applies to both.

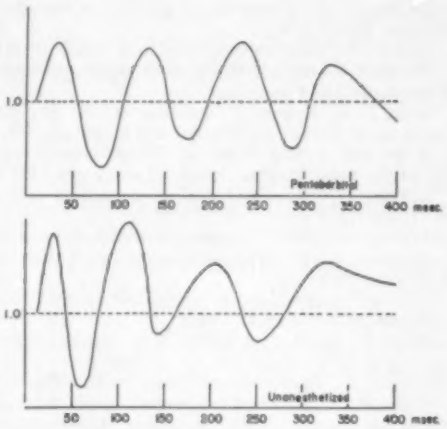

Fig. 6 (Marshall). *Upper curve.* Implanted electrode preparation under pentobarbital. Both eyes illuminated for one second starting at zero time on plot. Single shock applied to radiation neurons at intervals after start of illumination, and response recorded from striate cortex. Amplitudes of striate response (component C4 in Figure 2) to radiation shock plotted in arbitrary units on Y-axis.

Lower curve. Brief photic flash followed by test shocks as in upper curve but no anesthesia using implanted electrodes. Note similarity of the two curves. Length of photic flash not critical if bright enough to be effective in either case. Eyes dark adapted in both cases.

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Discussion

Fuortes: Thank you very much, Wade. We have heard Dr. Bishop's name mentioned several times during this symposium, but we haven't heard Dr. Bishop's voice as yet.

Bishop: I have nothing to add to Dr. Marshall's account of the visual system response except to ask, where do we go from here? A number of people have now obtained still more complicated responses to visual stimulation than one obtains from a single shock to the optic tract. To start with the optic nerve discharge; in the rabbit a brief flash of light to the retina is followed after a short delay by a series of nerve responses which show at least two maxima, finally followed by an off response if the flash is sufficiently long. In other words, there are two nerve pulses to the turning on of a light. These two are also represented in the geniculate response, but are summated to a single slow diphasic wave in the cortex. The nerve response is like the record from the frog we saw yesterday, or like that of the chicken as picked up at the colliculus level. This all occurs within a relatively short time, there is only a 10 msec. delay between the first two nerve maxima, and not over 15 between these and a third that may appear. The time is too short to correspond to any second cortical event.

Orrego, working in Chile, and now in our laboratory, and, I believe, Crescitelli, have recorded following the single summated diphasic specific response of cortex, a further response not corresponding in time relations to any event in the optic tract or geniculate. It occurs at a latency of about 40 msec. and is distinct from the later, often repetitive cortical after-discharge that follows a single electric shock to the optic tract, and which has the character of an alpha train.

This extra wave, of strictly cortical origin, apparently consists of two parts, differently affected by the color of the flash against the background adapting illumination. During adaptation to blue light, a green flash results in an accentuation of the first component of the wave, while a red flash accentuates the second component, each flash reciprocally reducing the other component, over a wide range of intensities.

In other words, although the cat, according to psychologic tests is color blind, it has a difference in reaction to different wave lengths of light that are only detectable at

the cortical level, and which imply different paths to cortex not detectable in the nerve record, or by electric stimulation.

Now there is nothing inconsistent in an animal having a long range of wavelength reception and even more than one receptor mechanism to cover that range, and still lacking color discrimination. A wide range of wavelength reception perhaps requires two kinds of pigment and two kinds of receptors to handle it, and they may have separate paths to handle it. We presumably "see" not with the optic projection cortex but rather in other areas of the brain to which this area relays its information, where it may or may not be resolved in terms of color. The point is that here is a cortical pattern to light stimulation more complex than to electric shock stimulation of the same nerve path, and with implications not predictable from the responses to electric stimulation or to simple light flashes.

Well, Dr. Marshall has shown some of the differences between effects at the cortex of light flash stimulation and of electric shock stimulation. The synchronized impulses in the nerve following one shock result in the exhibition of the maximum of detail in the cortical record, permitting the assignment of cortical activity to certain groups of neurones and even to different parts of each neurone. The response to the simplest flash is a dispersed or barrage pattern in the optic nerve, instead of a synchronized volley, and in the resultant cortical record most of the details are confused and smoothed out to indistinguishability, but some events can be inferred by comparison with single shock records.

With only one more complication, wavelength differences, still further confusion is introduced, still less resolvable in terms of simpler responses. Most of the responses from the visual cortex so far employed follow such strong stimuli, to show sufficiently categorical results, as to be practically nonphysiologic. And we have hardly begun to consider the sequelae of pattern vision, contrast, movement, and so forth, anywhere except at the retina. In dealing with cortical response patterns we have been performing, so far, as mechanics rather than as physiologists.

How can we steer between Scylla and Charybdis, between volley responses too simple to be significant, and normal behavior patterns too complicated to be soluble?

Other techniques must be focused on these problems. Single unit recording may prove to be one of the promising techniques. Tasaki has shown, in comparing single cell responses following electric stimulation to the over-all response pattern of cortex to the same shock, that cortical neurones may fire anywhere within the total recordable envelope, or even outside it. This looks like one promising place to dig. The cortex, however well we know the input from the retina, is organizing its own pattern further at its own level, depending in complex ways on not only the details of its own sensory input but on other concomitant cortical events and behavior patterns.

When we have resolved some of these complications, we may be in a position to inquire how a visual sensation is elaborated.

FUORTES: Thank you very much, George. I see Dr. Grundfest wants to speak.

GRUNDFEST: I want to add some information which is confirmatory of the nice results by Dr. Marshall, and which, perhaps, may contribute also some explanatory data. Very small doses (as low as 4.0 mg./kg.) of Nembutal injected into unanesthetized, succinylcholine-paralyzed cat preparations affect very profoundly the cortical activities evoked by stimulation of a number of subcortical pathways (Purpura, D. P.; In: Third Conference on Neuropharmacology, Josiah Macy, Jr., Foundation, New York, 1957; International Symposium Reticular Formation of the Brain, Detroit, 1957, in press; Grundfest, H.: In: International Symposium Reticular Formation of the Brain, Detroit, 1957, in press; Electrophysiology and Pharmacology of Dendrites, EEG & Clin.

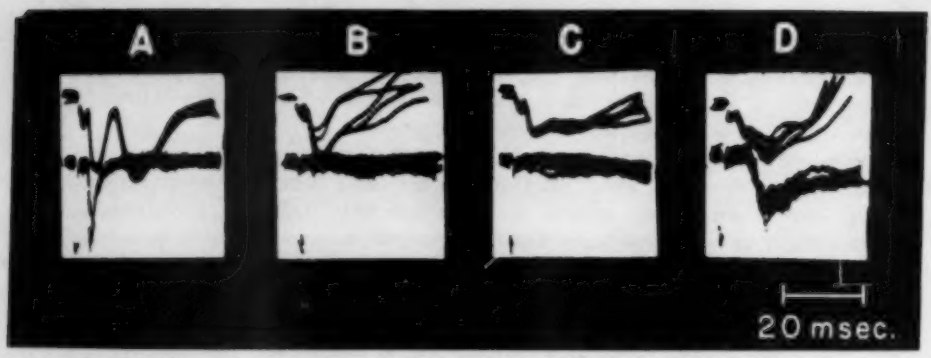

Fig. 7* (Marshall). Effects of 10 mg./kg. Nembutal upon thalamically induced cortical responses in unanesthetized, succinylcholine-paralyzed cat preparation. Simultaneous recordings from cerebral cortex (upper trace) and pyramidal tract (lower trace). Stimuli were delivered to specific thalamic nuclei at two-second intervals. Each record shows five superimposed responses. (A) Sequence of control records showing the regularity of the responses, a predominantly surface-positive sequence of electrocortical potential and an early, brief discharge in the pyramidal tract (latency two to three msec.; duration about six to nine msec.). (B-D) Sequence of consecutive recordings begun about 10 seconds after intravenous administration of 10 mg./kg. Nembutal. (From unpublished data by Purpura, Girado and Grundfest.)

Neurophysiol., in press; Purpura, Girado, and Grundfest, in preparation).

For example, when the thalamic pathway of the augmenting response is stimulated at a low rate (once very two seconds) the initial electrocortical activity is a very reproducible sequence of surface positive potentials seen in the upper trace of Figure 7-A. As is well known, these infrequent stimuli do not evoke the marked dendritic surface-negativity whose augmentation with more frequent stimulation has named this response. The same stimulus also evokes an early and brief discharge in the pyramidal tract (fig. 7-A, lower trace).

Upon administering 10 mg./kg. Nembutal (i.v.) the cortical potential began to change, the records in *B* and *C* each showing the superimposed traces of five responses in a 10-second interval. During the 20-second period the pyramidal tract discharges were suppressed. Then (fig. 7-D) the cortical responses to the single stimuli took on the form which is seen characteristically in the repetitively evoked augmenting responses, a sequence of surface positivity followed by large surface-negativity. Associated with this change is that in the character of the corticospinal activity. The pyramidal tract discharge became delayed and greatly prolonged. This, too, is the type of tract activity seen in the augmenting responses (Brookhart, J. M., and Zanchetti, A.: EEG & Clin. Neurophysiol., **8**:427-444, 1956).

As an isolated observation, the effects shown in Figure 7 would be difficult of analysis. However, other data give clues to the processes that must have been affected by the administration of the drug. Figure 8 shows the first four responses at the cortex and in the pyramidal tract when stimulating the thalamic pathway in the unanesthetized, succinylcholine-paralyzed preparation at eight/sec., the rate at which augmenting responses are best elicited.

The sequence of cortical potentials and the pyramidal activity evoked by the first stimulus were essentially identical with the responses seen on a different time scale in Figure 7-A. The next stimulus, arriving 125 msec. later, evoked a different pattern of potentials. The early cortical positivity was eliminated and the large dendritic surface-negative "augmenting" potential arose out

* Figures 7, 8, and 9 were presented by Dr. Grundfest.

of the enhanced late positivity. Onset of corticospinal activity was also delayed, but the discharge now became larger and very prolonged. At the third response the new pattern was stabilized.

This sequence, induced by repetitive stimulation, thus duplicates the pattern of effects caused in the single responses by a small amount of Nembutal. In both cases effects are ascribable to a sequence of synaptic processes both excitatory and inhibitory, the drug tending to accentuate processes that occur but are hidden in the single responses of the unanesthetized preparation.

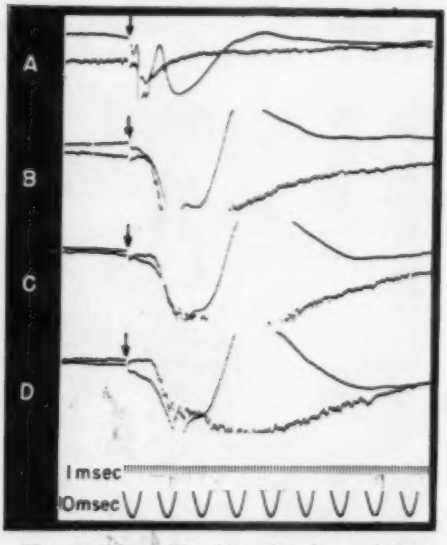

Fig. 8 (Marshall). Alteration in cortical responses produced by repetitive stimulation of specific thalamocortical "augmenting" pathway at eight/sec. Simultaneous recordings from cortical surface (upper trace) and pyramidal tract (lower trace) in unanesthetized, succinylcholine-paralyzed cat preparation. The first stimulus (shock artifact indicated by arrow) evoked a sequence of surface-positive responses in the cerebral cortex, terminated by a small surface-negativity. An early, brief discharge was evoked in the pyramidal tract. These activities are essentially identical with those in Figure 7-A. The next stimulus, delivered 125 msec. later, evoked markedly different responses at both recording sites. These became stabilized at the third stimulus. Note similarity of these responses with those in Figure 7-D. (From unpublished data by Purpura, Girado, and Grundfest.)

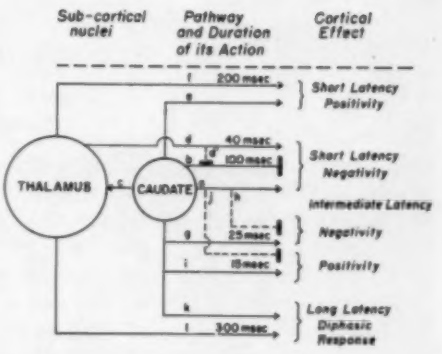

Fig. 9 (Marshall). A diagrammatic representation of excitatory and inhibitory activities initiated in the cortical pathways by a single brief stimulus to the head of the caudate nucleus. Lines terminating in arrows represent excitatory pathways, those terminating in bars indicate inhibitory action at the cortex. Broken lines indicate probable paths of synaptic activity. Times, where given, indicate approximate duration of the synaptic effects with respect to specific varieties of cortical activity that the caudate stimulus is capable of evoking. (Purpura, Housepian, and Grundfest, Arch. ital. Biol., in press.)

The responses evoked by other corticipetal pathways also exhibit effects of drugs or of repetitive stimulation, the specific actions depending upon the synaptic organization of the system. Indeed, the changes produced either by repetitive stimulation or by drugs may be used to analyze synaptic organization.

As an example, Figure 9 shows the various corticipetal pathways put into motion by a single stimulus to the head of the caudate nucleus. This stimulus evokes only a rather brief response in the cortex, and only in a restricted region of this. Yet, inhibitory and excitatory activities lasting some 300 msec. are developed which are not manifested in the single response, but which can be demonstrated by appropriate tests (Purpura, D. P., Housepian, E. M., and Grundfest, H.: Arch. ital. Biol., in press).

CORTICAL UNIT RESPONSES TO VISUAL STIMULI IN NONANESTHETIZED CATS*

DAVID H. HUBEL, M.D.
Washington, D.C.

This paper represents the beginning of an attempt to improve our understanding of the nervous system by studying normal activity in single cells. The approach we have employed involves recording from single units in the unanesthetized unrestrained animal. We have done this in order to examine discharge patterns in various states such as wakefulness and natural sleep, and to influence these patterns, if possible, by physiologic stimuli. We have thus hoped to combine the advantages of two important recent advances in electrophysiology, the development of methods for the chronic implantation of electrodes on the one hand, and the use of microelectrodes on the other. We have felt that such a combination might be especially promising in studies of the higher central nervous system, where such things as anesthetics, brain stem sections, and paralyzing drugs are most likely to distort normal neuronal discharges.

The specific aim of the present project has been a survey of unit activity in the cat's visual cortex. The only other microelectrode survey of this region with visual stimuli is that of Jung and co-workers.[1,2] This was carried out with the encéphale isolé cat and made use of binocular stimulation with a diffuse light source. By employing prolonged light flashes they were able to differentiate three types of units activated respectively by on, by off, and by both on and off.[3] These were termed b, d, and e units. A rare type, c, was inhibited by both on and off. About half of the units they observed did not respond to diffuse illumination: a major problem at present is that of determining the role or roles played by these "a units" in visual processes.

I should like now to outline briefly the methods used in this research (fig. 1). Several days before the first recording from a given cat a hollow plastic (Kel-F) peg is securely threaded into the animal's skull. At the time of recording a hydraulic piston-cylinder assembly holding the microelectrode is threaded onto this peg. Oil introduced into the cylinder causes the piston and hence the electrode to be lowered until unit responses

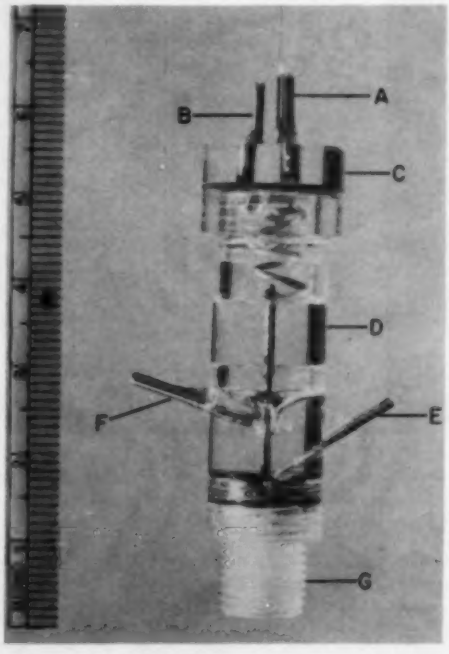

Fig. 1 (Hubel). Photograph of piston-cylinder assembly threaded in place onto the Kel-F implant. (A) Oil inlet. (B) Connector for microelectrode. (C) Cylinder lid. (D) Piston. (E) EEG terminal. (F) Oil outlet. (G) Kel-F implant. The EEG terminal makes contact with a metal sleeve which surrounds the electrode and whose lower end rests in spinal fluid close to the surface of the cortex. This sleeve serves also as a guard to protect the electrode when the cylinder is attached to the implant.

* From the Department of Neurophysiology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center.

are found. Since the electrode is, for a given piston position, fixed with respect to the skull, all but very violent head movements may be made without loss of a unit.

The electrodes, which were especially developed for this work, are electropolished tungsten wires coated with a vinyl lacquer. Their main advantage is their strength, which allows them to penetrate pia whether normal or thickened, and also dura.

The cat is held in a chest harness, with the head entirely free (fig. 2). He faces a large white semicircular screen which may be lit either diffusely by a distant source such as the room lights, or by a small circular spot. This subtends an angle of about two degrees at the cat's eye, and, in brightness, measures about 1.0 meter Lambert. It is produced by a flashlight several feet away, which may be set to swivel vertically or horizontally. The process of swivelling changes the setting of a potentiometer, which in turn shifts one of the oscilloscope beams, providing an indication on the record of the spot's position. For this method of stimulation to be useful the cat must be alert, but not so interested in the spot that he follows it with his head and eyes. Luckily a cat usually only does this for a few minutes until his interest apparently wears off, after which he may just stare lazily at the screen for several minutes at a time without moving his eyes.

For recording we chose the center of the lateral gyrus at Horsley Clarke frontal plane zero. In all cases brains were examined histologically to verify that the recording site was striate cortex. In a few cases electrolytic lesions were made: one of these will be shown later. It should be emphasized that, except where lesions have been made, our knowledge of the depth of any unit is uncertain, and that some of the records may have been made from fibers in subcortical white matter, that is, from afferent fibers or axons of cortical cells. Presumably all records from cell bodies have been extracellular.

Over a period of one year, 300 units have been isolated and studied for periods ranging from several minutes to several hours. As the goals of this project have become better defined we have made increasing efforts to obtain three different types of information about each unit. The first of these, as yet the most important, is the stimulus which is most effective in influencing the unit's firing. This will be discussed more fully later. The second is the layer of the cortex in which the unit is situated. We have come to realize the futility of tackling this problem except by histologic means, but our techniques for doing this have been so recently developed that as yet very few units have been accurately located. The third is the spontaneous activity patterns which the unit exhibits. Three examples of these are shown in Figure 3. Here we make no mention of the animal's waking state or the presence or absence of light.

Fig. 2 (Hubel). Photograph of cat with implant over right visual region. Micropositioning assembly is in place. The implantation on the left is for surface EEG recording.

The first line shows a type of firing which is not, perhaps, completely random, but

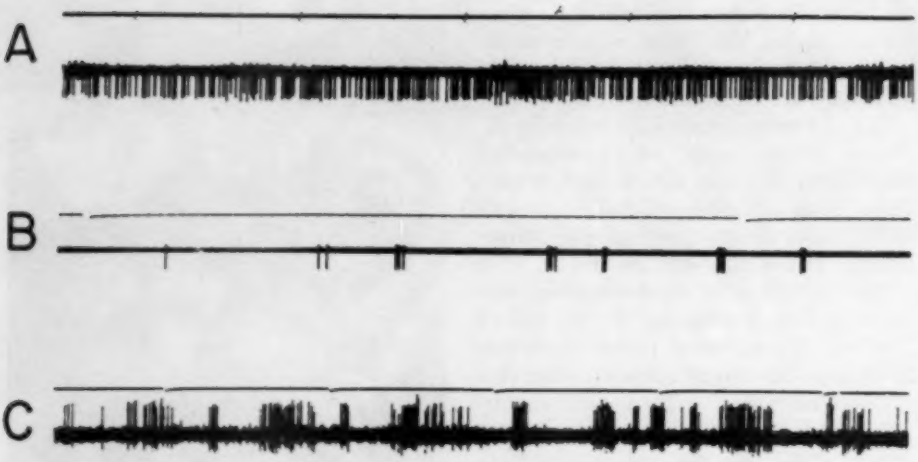

Fig. 3 (Hubel). Three examples of "spontaneous" activity patterns. (A) Ungrouped. (B) Clustered. (C) Bursty. For each example, upper beam shows time in seconds. Duration of time pulse 10 msec. Lower beam shows microelectrode record. Positive downward. Time constant 0.5 msec.

which certainly is not rhythmic. We shall refer to this activity as "ungrouped." The firing rate may be rapid, as in this example, or slow. In strong contrast with this is the second line, which shows a tightly grouped activity. which would be classed as a slow ungrouped rhythm were it not that single spikes are replaced by clusters of two to five or six, firing at frequencies reaching, at times, well over 500/sec. We shall term this activity "clustered." The third line shows still another very different pattern, which we term "bursty." It consists of highly irregular longer-lasting bursts of activity separated by irregular silent periods.

In most penetrations, examples of all of these modes of firing are seen. Some units show only one pattern. In others visual stimulation or change in waking state results in a shift of the prevailing pattern from one type to another. Since such a shift may be the most striking response we have felt it worthwhile to stress this concept of patterns at the outset.

Of the 300 units observed, 107, or about one third, were found to respond in a highly characteristic manner to diffuse light. For the sake of brevity we have provisionally called these *Class I*. Their four main features may be outlined as follows:

1. They respond in a consistent and definite manner to changes in diffuse illumination.

2. Their responses show adaptation which, while differing from unit to unit, in general has a time course of the order of seconds, that is, they do not, typically, respond to on or off by a very brief burst of only several spikes.

3. Their responses to light allow them to be categorized as "on," "off," or "on-off"—categories probably equivalent to those described by Jung[1] as "b," "d," and "e," respectively.

4. During the alert waking state their firing pattern is of the "ungrouped" type shown in the first line of Figure 3. With increasing drowsiness and depth of sleep, however, there arises an increasing tendency toward clustering of spikes shown in the second line. These clusters tend to be abolished by a change in illumination which would, with the cat alert, increase the unit's activity. On the other hand, they predominate when the unit would ordinarily be least active. For example, Figure 4-A shows

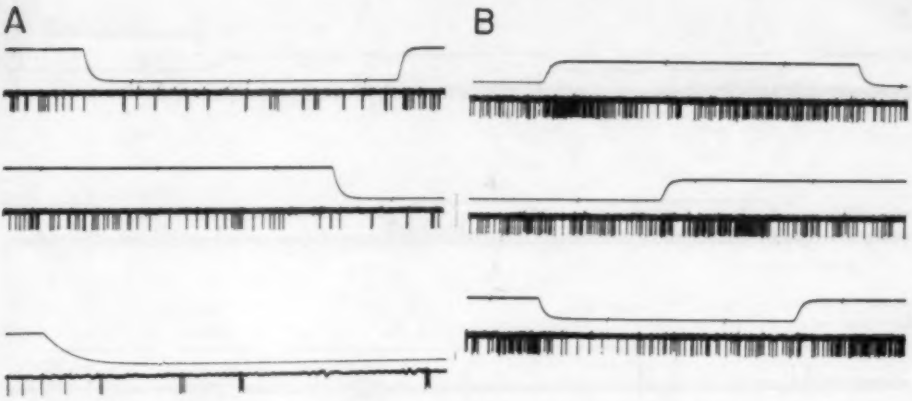

Fig. 4 (Hubel). Record of typical "on" unit, Class I. (A) Animal asleep. (B) Animal alert. Continuous records except for last line of A, which shows clusters with an expanded time scale. Upper beam, photoelectric cell recording of illumination; time, one sec., pulse duration 10 msec.

a record of a unit of the "on" type taken with the animal asleep. Clustering is present, and is clearly maximal at that time when the unit would ordinarily be least active; that is, during the off period. In Figure 4-B, the animal is alert, and clustering has disappeared. Figure 5 shows the converse of this in an "off" unit. Here the animal is asleep. Clustering is maximal following on, when an "off" unit is typically inhibited. You can see that here, for instance, the replacement of single spikes by clusters is the most conspicuous response to "on."

These, then, are some of the features of what we have called Class I units. It should be pointed out that they are not the only units which respond to diffuse light. Very rapidly adapting "on," "off," and "on-off" units are common: so far they have not been carefully studied.

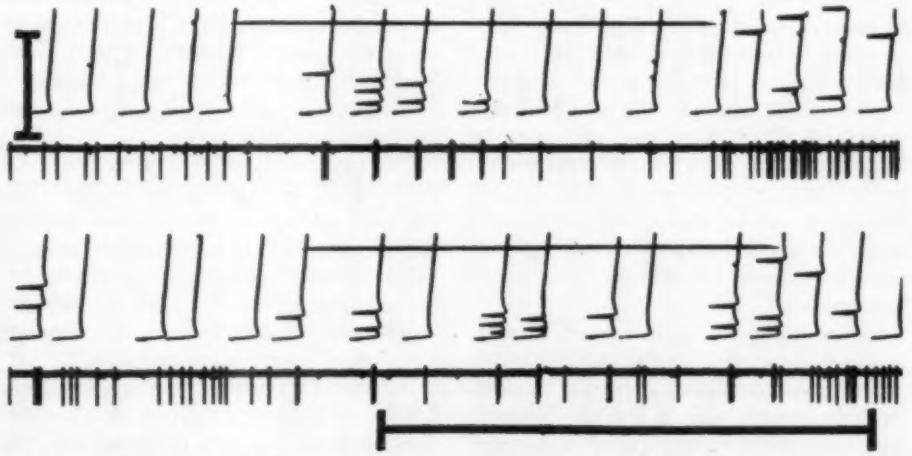

Fig. 5 (Hubel). Record of typical "off" unit, Class I. Light on and off, shown by upper horizontal beam. Continuous recording. Cat asleep. Vertical sweep is triggered by spikes, reads from below upward. Time: horizontal beams, one second; vertical sweeps, 20 msec.

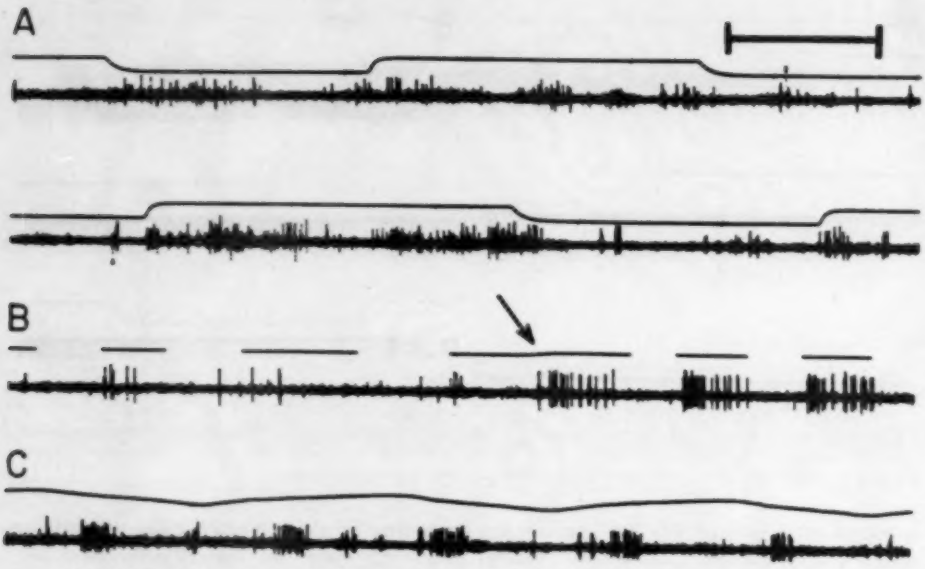

Fig. 6 (Hubel). "On" unit activated in a restricted region. (A) Diffuse light stimulation. Upper beam as in Figure 4. Note bursty firing pattern. (B, C) Restricted spot stimulation. Upper beam shows whether spot is on or off; displacement of this beam upward signifies shift of spot to left. The arrow indicates a slight shift of the spot's position to the left. Time: one second.

The remainder of our collection is made up of units which gave no response, or only vague disputable responses, to diffuse light. These comprise about half the group. We have found that these units are by no means all unconcerned with visual processes, since in many cases simply passing one's hand back and forth in front of the cat's face evokes a brisk response. We have been able to analyze 12 of them more accurately by using a small movable spot of light trained on a screen in front of the cat. By this method it can be shown that many units which are not activated by diffuse light are exceedingly sensitive to light in a highly restricted area.

Figure 6 shows a typical record. The top two lines show the failure of diffuse light to produce activation. We note that in this case the firing pattern is of the irregular "bursty" type, quite unlike the ungrouped or clustered activity seen in Class I units. Spot stimulation is shown in the third line. At first little or no response is seen to turning the spot on and off, until it is moved about one-half inch (two degrees) as shown by the small shift in the upper beam. When this is done intense on activation of the unit occurs. In the bottom record the light is kept on while the spot is moved back and forth over the sensitive region. Here a burst of firing occurs at each crossing, regardless of the direction of movement.

The converse type of unit, activated by off, is shown in Figure 7. In the upper line the spot, trained on the sensitive area, is blinked on and off. In the lower line we again see the effect of going back and forth over the sensitive region. This time the response is asymmetric to such a degree that only one direction of movement is effective.

An interesting variant is shown in Figure 8. The unit illustrated here was clearly sensitive to stimuli in a very restricted area, but in this case the spot had to be moving, since simply turning the light on and off had no

effect. The first four lines show records of responses to movement across the sensitive region in a right-to-left direction. At times there is a hint of a response for the reverse direction. In the bottom record the light moves up and down, producing brisk responses to downward movement.

A final example of a similar sort is shown in Figure 9. The upper line shows the absence of any clear or sustained response to diffuse light, though against the bursty background there is a suggestion of a brief response at on and off. In the second line we see a brisk response to a horizontal movement to the left, carried out in the animal's contralateral visual field. In the third line no response is seen for a similar stimulus in the ipsilateral half field. The remaining lines show a continuous record taken a few minutes later, by which time a second unit had come within range of the electrode. This unit, in contrast to the first, is activated by

Fig. 7 (Hubel). "Off" unit activated in a restricted region. (A) Light blinked over sensitive region. (B) Light moved back and forth over sensitive region. Time: one second.

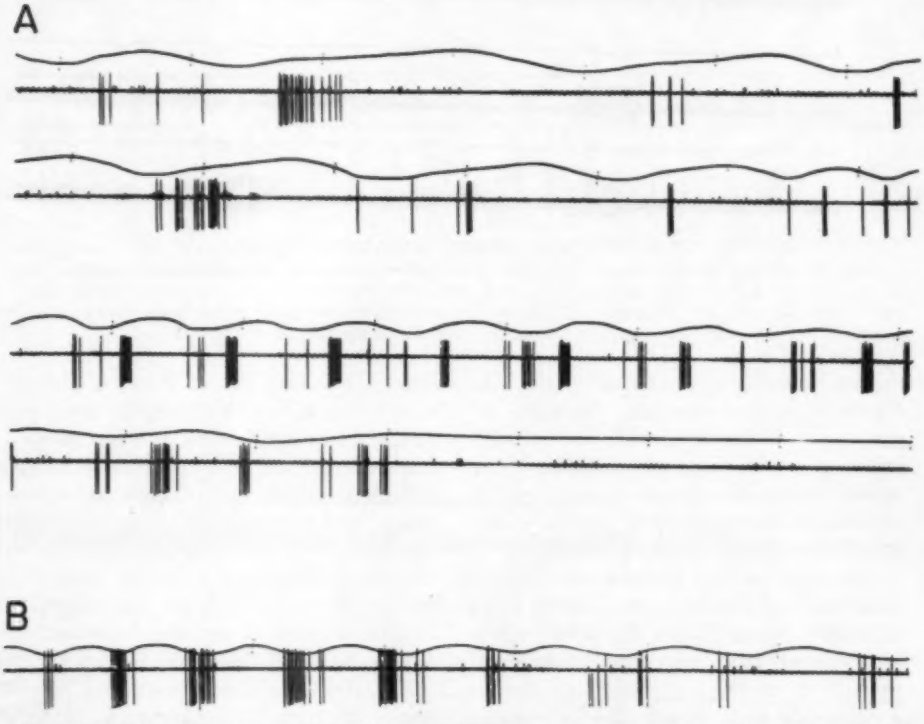

Fig. 8 (Hubel). Unit activated by moving spot but unaffected by turning of a stationary spot on or off. (A) Horizontal movement over sensitive area: upward deflection of upper beam represents movement to the left. (B) Vertical movement over sensitive area: upward deflection of upper beam represents upward movement. Time: one second.

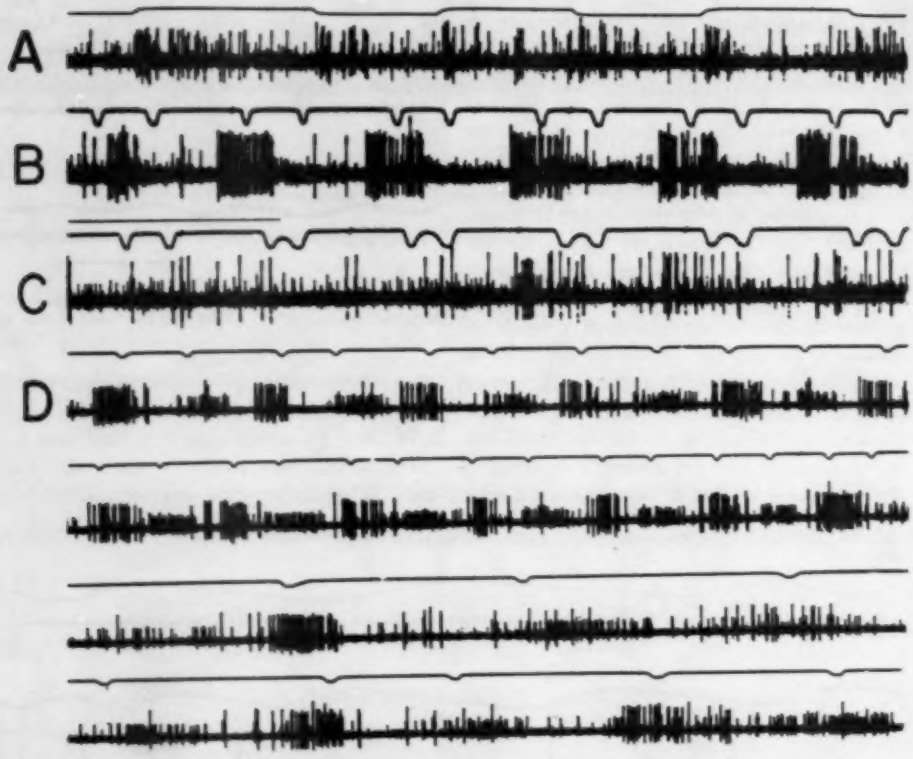

Fig. 9 (Hubel). Units activated by slow horizontal to-and-fro hand movements of about two to three degrees. Note bursty firing pattern. (A) Stimulation with diffuse light. (B) Movement in left (contralateral) visual field. (C) Movement in right visual field. (D) Movement in left visual field, several minutes later. Upper beam: (A) Photocell. (B, C, D) Monitor improvised by allowing hand shadow to cross photocell once for each half excursion. Time: one second (visible only on B and C).

the reverse movement, to the cat's right. Like Figure 8-A, this example illustrates responses to movement which are strongly preferential with respect to direction. It also shows that the two classes of units, one responding to movement to the right, the other to movement to the left, are intermixed.

Though as yet only 12 units showing these restricted responses have been studied with a light spot, several times this number have been demonstrated with small moving objects. Even at this early stage we can see a suggestion of a second class of units—a suggestion which is supported in several other ways. For example, most or all of these units have the bursty irregular firing pattern described above. We have also observed that units with this bursty firing pattern are seen consistently at a certain early stage of each penetration. Moreover, it is difficult to isolate one of these units from others, and simultaneous records of several unisolated negative units are frequent. In these ways they differ markedly from Class I units which fire with an "ungrouped" or "clustered" pattern, are easy to isolate, and tend to be found deeper in the cortex.

The following slides illustrate a possible means of getting more anatomic information. At a depth of roughly four millimeters the unit of Figure 10-A was observed. The record is typical of a Class I "on" unit in

the sleeping animal. Here an electrolytic lesion was made by passing 10 microamperes through the electrode (negative) for 10 seconds. The electrode was then pulled back, and during withdrawal we passed the region of hard-to-isolate bursty units already encountered on the way down.

Figure 10-B shows a record of this activity, with the absence of a response to diffuse light. A second lesion was made here, and the electrode was pulled out. The brain was then perfused and sectioned serially, and in Figure 11 we see a Nissl-stained section through the area.

The first part of the electrode track is seen leading to the more superficial lesion, the one made on the way out. This turns out to be in layer IV (fig. 12). Far below, in layer V and close to the border of layers V and VI, is the deeper lesion. A higher power view in Figure 13 shows the very characteristic morphology of the deeper lesion. We see the onionlike shape with a clear core surrounded by a dark staining area surrounded in turn by a pale region—which makes it very easy to find and recognize these lesions. Clearly we will need dozens of examples like this before conclusions can

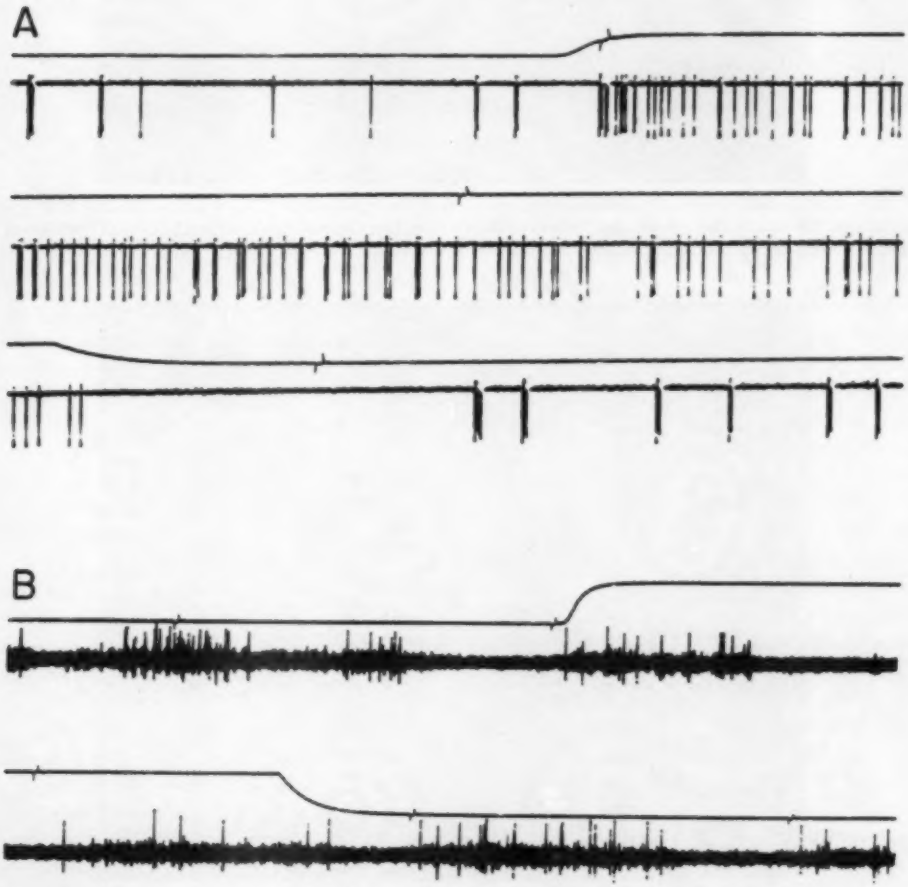

Fig. 10 (Hubel). Units corresponding to lesions shown in Figures 11 to 13. (A) Class I "on" unit response to diffuse light, in sleeping state. (B) Bursty unit activity. Time: one second.

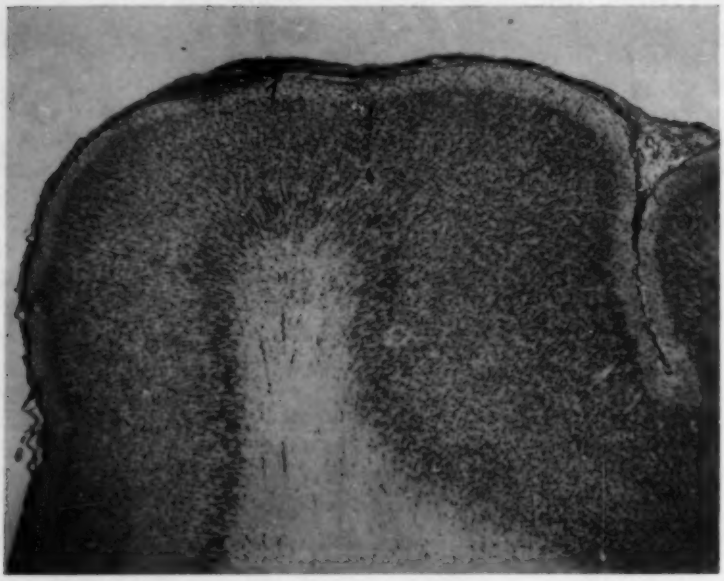

Fig. 11 (Hubel). Photomicrograph of frontal section through lateral gyrus (×18). Midline is to left of figure. Electrode track is outlined by inflammatory cells from beginning of layer II down to first lesion. Second lesion is at about 2.5 times the vertical depth of the first.

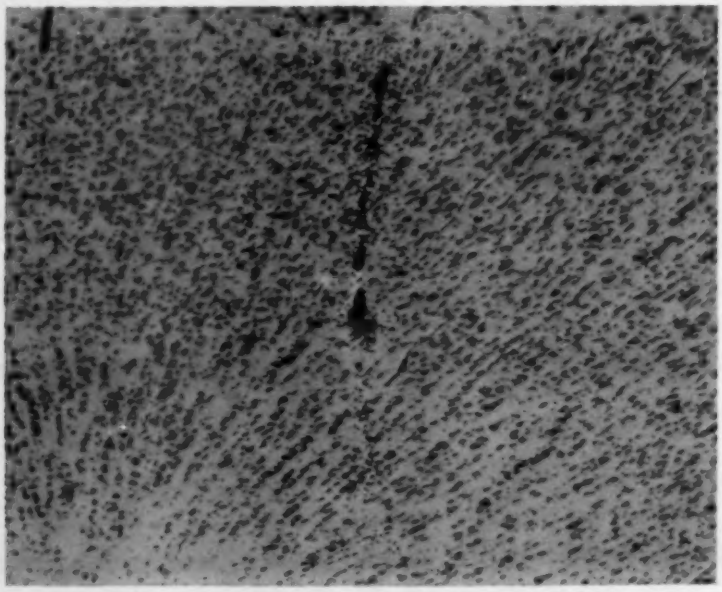

Fig. 12 (Hubel). Higher power view of the superficial lesion (×64).

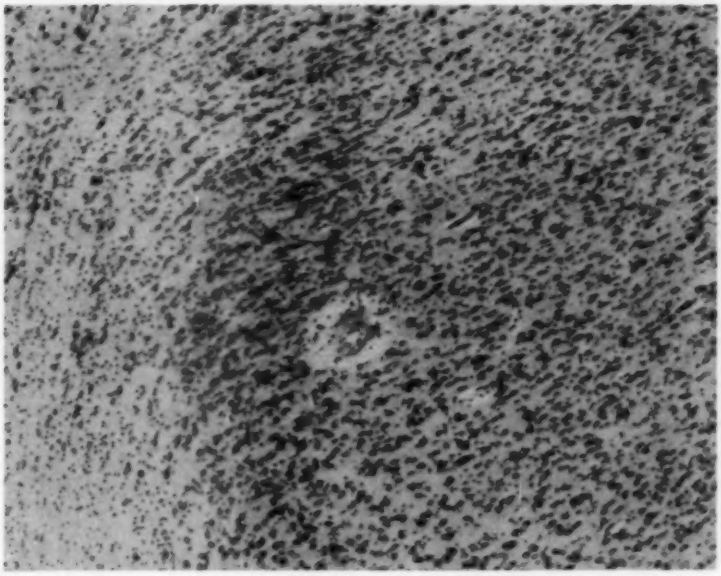

Fig. 13 (Hubel). Higher power view of the deeper lesion (×64).

be drawn, but the method seems promising.

To return to responses to a restricted spot: we have defined Class I units by four criteria, one of which specifies brisk responses to diffuse illumination; we have also described another group of units which responds only to restricted spots of light. So far nothing has been said about responses of Class I units to these restricted spots. In the few cases where the experiment has been tried, and these include both "on" and "off" units, the results have been unequivocal.

Figure 14 shows a typical "on" unit, as we can see from the diffuse light responses in the first line. In the next two lines there is a symmetric response to a moving spot, whenever the sensitive region is crossed. In the fourth line the light is kept on and moved abruptly from the sensitive region to points about one-half inch (two degrees) to either side, and back. Finally, in the last line the light is blinked on and off while being moved back and forth. These results, then, show restricted on activation. Analogous results have been seen for the off unit. To sum up, the Class I unit seems to be driven by light from a restricted sensitive region in the visual field.

We have yet to explain why, for our second class of units, diffuse light is virtually ineffective. Though to date no direct tests have been made, a good guess would be that for these units the sensitive region of the retina is surrounded by an area which interacts with it in an opposing manner. Such inhibitory surrounds have been well worked out in the cat's retina by Kuffler,[8] and to find them re-represented at the cortex would not be surprising. They could explain not only why some units respond to a spot but not to diffuse light, but also why movement across the sensitive region is often more effective than simple blinking. Finally if some surrounds were asymmetric we might account for the asymmetric movement responses seen in some units (figs. 7, 8, and 9).

To conclude I should like to show an example of a group of units which seems to have different properties from either of the

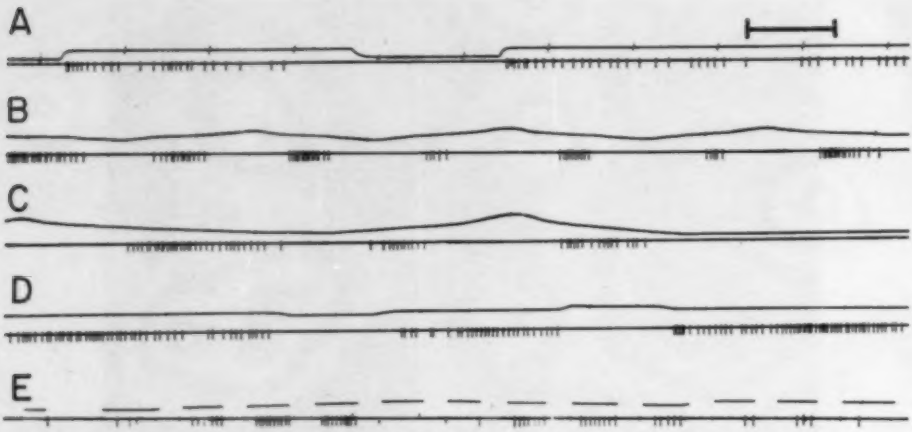

Fig. 14 (Hubel). "On" unit, Class I. (A) Responses to diffuse illumination. Animal is alert. Note ungrouped firing pattern. (B-E) Responses to restricted spot illumination. Upper beam shows whether spot is on or off; displacement upward signifies shift of spot to left. Time: one second.

above groups. These show activation by horizontal movement in one direction only, but, unlike the examples shown above, where the region of activation was highly restricted, there is a strong suggestion that they are driven throughout a wide region of the visual field. Activation may be produced by either a spot of light or an object, so long

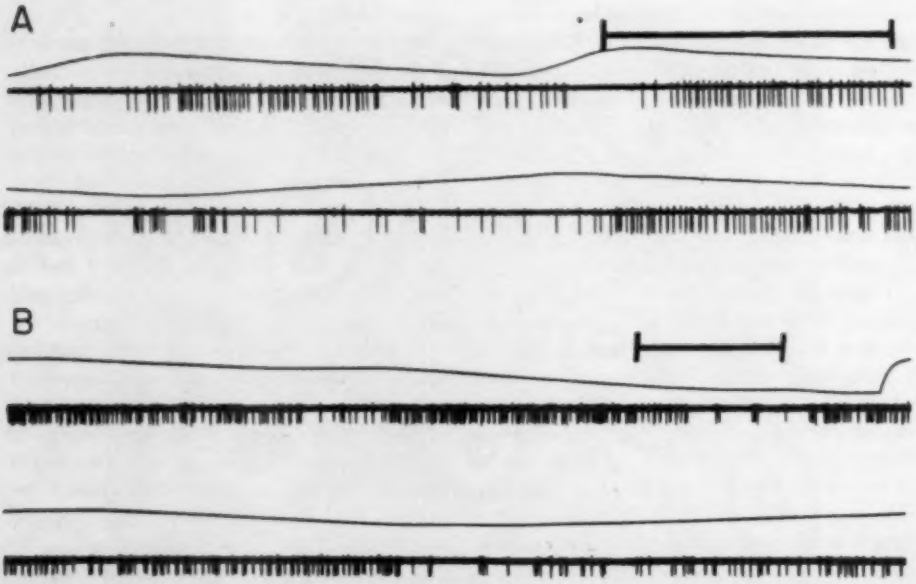

Fig. 15 (Hubel). Unit responding to movement (see text). (A) Responses to horizontal movement of spot. (B) Responses to rotating drum (deflection at end of upper line of B represents resetting of potentiometer). Time: one second.

as it moves in the direction to which the unit is sensitive.

An example of one of these is shown in Figure 15-A. The upper beam shows the relative horizontal position of the spot, while the lower shows the responses. Here the unit is activated by movement to the animal's right.

Uninterrupted activation of such a unit is not possible, of course, with this type of to-and-fro stimulation. Accordingly we built a drum 12 inches in diameter and free to revolve on a vertical axis, and on it painted 32 equal alternate black and white vertical stripes. When this is rotated it turns a potentiometer in such a way that a decline in the first beam of the oscilloscope indicates movement to the cat's right. Using the drum we found that these units could indeed be driven as long as one wished. Figure 15-B illustrates activation produced by movement of the stripes to the right.

In closing it might be said that this survey of a few hundred visual cortical units has given a brief, almost tachistoscopic, and doubtless very incomplete view of visual cortical function. But along with neuroanatomic and behavioral data it may at least emphasize the vast complexity which we face when we study it.

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Discussion

RATLIFF: I find Dr. Hubel's description of cortical elements which respond to changes in the intensity and location of the light stimulus most interesting.

First, let me say that I agree fully with Dr. Hubel's interpretation of the role of inhibitory mechanisms in the generation of these responses. In fact, I am inclined to believe that the role inhibition plays in the generation of transient responses is probably of more importance for vision than is its role in brightness contrast, color contrast, and other related phenomena.

I would like to make a few comments on the visual significance of responses to stimulus changes, particularly those changes due to motion. Their importance for vision can best be demonstrated by arranging conditions so that no movement of the stimulus can occur. As you know, our eyes are continually in motion even when we attempt to fixate on a stationary object—these movements must be abolished, or compensated for, in order to produce a stationary image on the retina.

The slide (fig. 16) shows the technique for providing a stationary image on the retina which Lorrin Riggs, Tom and Janet Cornsweet, and I used a few years ago. Rays from a projector are reflected from a mirror on the eye to a screen and then viewed through a compensating path which is twice the length of the projection path. Under these conditions the image is stabilized on the retina. This stabilization occurs because, as

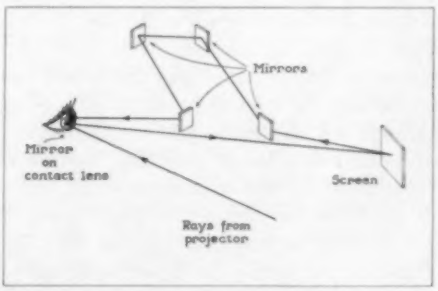


Fig. 16* (Hubel). Technique for providing a stationary image on the retina.

* Figure 16 was presented by Dr. Ratliff.

the eye moves, the projected beam moves an amount proportional to the eye movement.

Unfortunately the beam reflected from the mirror turns through twice the angle of rotation that the mirror turns through; but viewing the image through a compensating path which is twice the length of the projection path from eye mirror to screen exactly compensates for this so that the image is fixed on the retina. (Incidentally, this was studied quite independently of our work by Ditchburn and Ginsborg in England and also, somewhat later, by Toraldo di Francia in Italy.)

The most striking result of this arrangement is that any object viewed in this manner gradually disappears from view—all the contours and borders fade out within a few seconds, and the field appears perfectly homogeneous—even though the image on the retina is physically unchanged.

Now if you put a Dove prism in the return path, in such a position that the motion of the retinal image is doubled, then the image does not fade out. And a motionless image which has disappeared may be made to reappear simply by moving it on the retina. It appears, then, that motion, and the activation of those retinal and cortical elements which respond to motion, is absolutely necessary for vision.

I have discovered lately that you don't need all this gadgetry. There is a very simple way to produce a stabilized retinal image without any attachments to the eye. This is done by making use of the entopic phenomenon known as Haidinger's brushes. These may be seen quite easily by looking through a blue filter at a homogeneous field of polarized light. A sheet of Polariod in front of a fluorescent lamp about 10 inches or so from the eye works pretty well. If you look at the lamp through the filter and Polaroid you can see a figure-eight shaped image centered on the fovea. It is thought to be due to polarization effects produced by the orientation of molecules in the macula lutea. Since the macula lutea is fixed with respect to the retinal receptors, the location of the "brushes" is also fixed, and, under certain conditions, "Haidinger's brushes" are actually stabilized on the retina—just as is the retinal image obtained with the gadget illustrated.

The necessary conditions are that the plane of polarization of the light be fixed with respect to the eye—for the orientation of the brushes depends on the orientation of the plane of polarization. In the situation I have just described the image is stabilized because the brush will move around its center on the fovea only if there is either a rolling movement of the eye or a rotation of the Polaroid.

Now the eye doesn't roll during ordinary fixation—there's only an appreciable rolling when you make relatively large oblique movements—so that possible source of motion is taken care of. If you hold the Polaroid in a fixed position, the second possible source of motion is also eliminated, and you now have a stabilized image on the retina without any attachments to the eye and it fades out just as the one that I described before.

If you give the Polaroid a quick rotation, the brushes immediately pop back into view. This is probably one reason why many people have such difficulty in seeing Haidinger's brushes—by the time they get all the conditions right it has faded out due to the fact that it is stationary on the retina! You have to keep moving the Polaroid or rolling the eye in order to keep the brushes visible. Evidently, the responses to motion, described by Dr. Hubel, are of considerable significance for vision.

FUORTES: Thank you very much, Dr. Ratliff. I don't know if Dr. Hubel would like to comment on Dr. Ratliff's discussion.

HUBEL: Well, only to say that perhaps these mechanisms then are in existence to hide Haidinger's brushes, as it were.

FUORTES: Thank you very much. I have been told that coffee will be available at the cafeteria between 10:40 and 11:00—so anybody wishing to have coffee may do so now.

ANALYTIC STUDIES OF THE ELECTROMYOGRAM OF HUMAN EXTRAOCULAR MUSCLE*

GOODWIN M. BREININ, M.D.
New York

The role of electromyography in clinical diagnosis of neuromuscular disorders has been essentially qualitative. A simple display of the configuration of action potentials and of the muscle discharge patterns usually has been deemed sufficient.[1, 2] A comparison may be made with tissue pathology in that the former may be likened to the observation of individual cell types and the latter to the cytoarchitecture of tissues. Both aspects are important. The diagnostic role of electromyography is generally satisfied with the answers to these questions: Are the muscle potentials normal or abnormal in amplitude, shape, duration, and frequency? Are the discharge patterns normal or abnormal at rest and on sustained effort with respect to frequency and recruitment of motor units.

The study of neuromuscular physiology, however, requires higher degrees of quantification than are feasible with simple inspection of the trace. Numbers of investigators during the past several decades have applied electronic circuitry to problems of neurophysiology. Electroencephalography has been the chief field for utilization of such techniques as integration and frequency spectrum analysis.[3] Integration has also been employed in peripheral muscle[4, 5] and in extraocular muscle electromyography.[6, 7]

Innervational analysis is of great importance in kinesiologic studies of extraocular muscles. Through it a clearer understanding of motor control mechanisms of the brain is being achieved. The correlation of volitional and reflex innervation to these muscles with the resultant movements of the eyes and the effects of lenses, prisms, and drugs are the subjects of current investigations utilizing quantitative electronic techniques.[8–11]

QUALITATIVE ASPECTS

The qualitative or diagnostic data of extraocular muscle electromyography have closely paralleled those of peripheral skeletal muscle. The major modification of extraocular muscle consists of a smaller innervation ratio associated with motor units of smaller amplitude, shorter duration, and higher frequency of firing than peripheral skeletal muscle units. These distinctions have been abundantly documented in the literature. In addition, the extraocular muscles exhibit constant activity during consciousness and are completely silent or nearly so only during anesthesia or sleep. This is in contrast to peripheral skeletal muscle which is electrically silent at rest. The point is that the extraocular muscles are never at rest during the waking state. This difference in behavior reflects the difference in functional demand placed upon eye muscles, 12 of which must work in harmony to co-ordinate movements of the two eyes.

In disease of the neuromuscular apparatus precisely the same alterations of electric activity appear in both ocular and peripheral skeletal muscles (figs. 1, 2, 3, and 4).

Lesions of the neuron produce disturbances of motor unit configuration and firing pattern. Transection of a nerve gives rise to fibrillation of the denervated muscle. Fibrillation potentials are the small, brief discharges of single muscle fibers which occur spontaneously at rest and cannot be recruited. Such potentials are readily recognized in denervated peripheral muscle but are far less frequently seen in extraocular muscle. Nevertheless they do occur.

* From the Department of Ophthalmology of the New York University Post-Graduate Medical School. This study was supported by a grant (#B-911[C$_3$]) of the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland, and by grants from the Fight for Sight League of the National Council to Combat Blindness and the Stanley Tausend Foundation, New York.

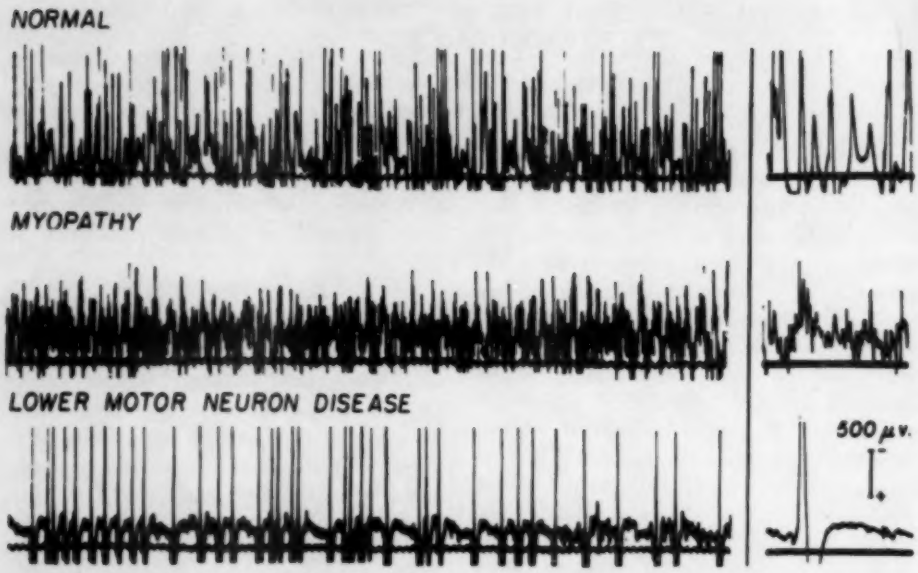


Fig. 1 (Breinin). Peripheral electromyogram (from Eaton and Lambert).

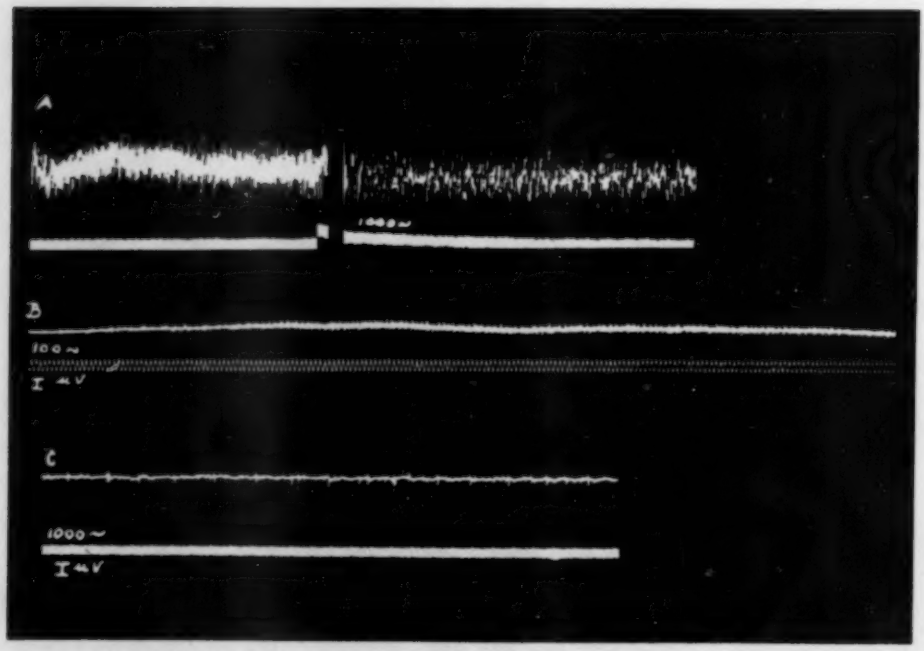


Fig. 2 (Breinin). Ocular electromyogram. (A) Normal medial rectus. (B) Myopathy. (C) Lower motor neuron disease.

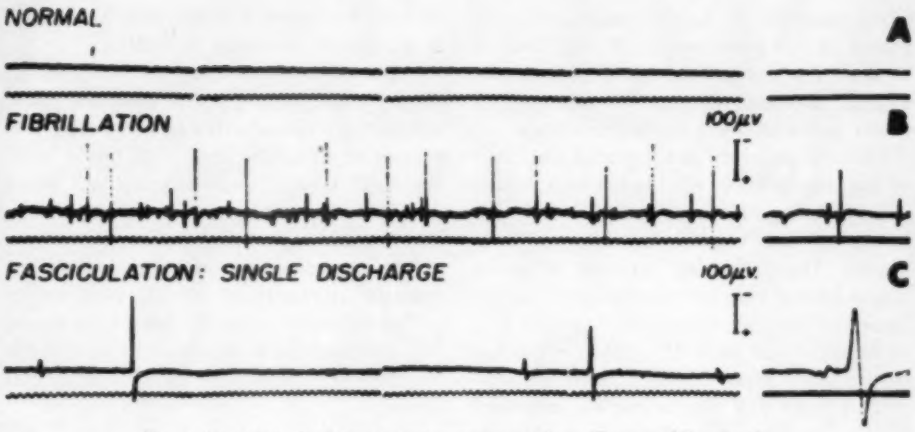


Fig. 3 (Breinin). Peripheral electromyogram (from Eaton and Lambert).

It is probable that compression block of an extraocular nerve occurs and gives rise to electric silence of the muscle. This has been demonstrated in peripheral nerve and has been shown by Denny-Brown to be related to focal demyelination.[2] Restoration of the myelin sheath is accompanied by renewed nerve conduction and electric activity of the muscle. The electromyogram in diabetic neuropathy of the extraocular muscles ex-

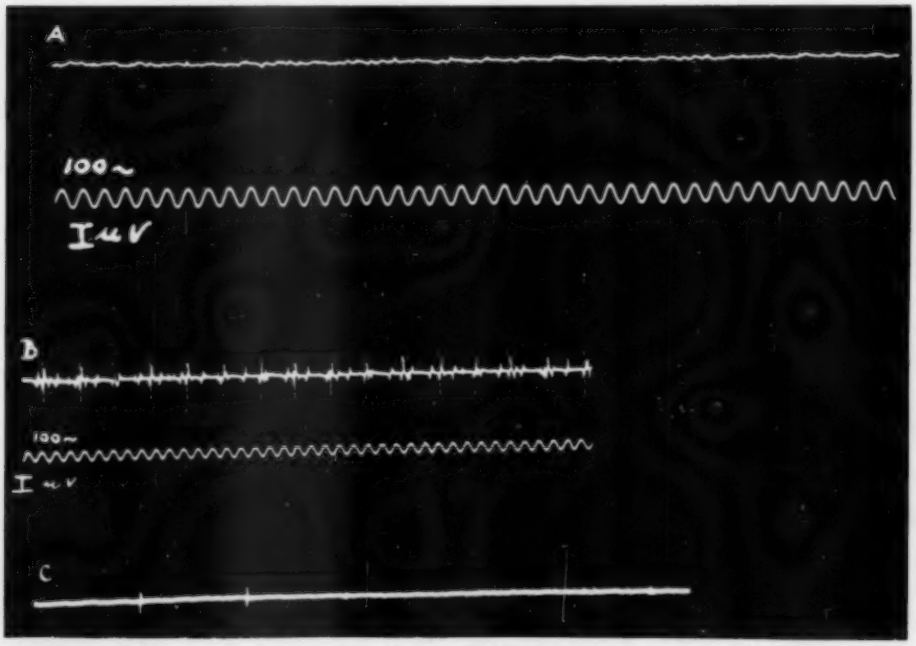


Fig. 4 (Breinin). Ocular electromyogram. (A) Neuropathy. (B) Fasciculation. (C) Fibrillation.

hibits complete or nearly complete electric silence at first but recovery of full function occurs in a matter of six to eight weeks without aberrant regeneration. The pattern fits in well with nerve block phenomena.

Disturbance of the synchrony of discharge of the muscle fibers of a motor unit results in polyphasic potentials. These occur in nerve lesions of both extraocular and peripheral muscle. The polyphasic potential of extraocular muscle closely resembles the fasciculations of peripheral muscle and may, in fact, be identical with them. Foci of nerve damage may disturb nerve accommodation with resultant repetitive discharges as postulated by Denny-Brown. Further experience may permit localization of the disturbance to the proximal or distal portions of the ocular nerve just as is possible in the case of peripheral nerves.

The innervational pattern on effort in neurogenic lesions shows the same loss of units and inability to sustain the level of discharge in both types of muscle.

Aberrant regeneration in third nerve palsy produces activity of most or all of the complex of muscles innervated by the nerve whenever it is fired. Some potentials are of large amplitude and long duration—reinnervation potentials; others are quite small.

Myopathic or dystrophic muscle is characterized by a loss of muscle fibers with retention of most of the motor units. This gives rise on effort to an abundant discharge of low voltage units some of which are polyphasic. The dystrophic pattern is similar in both types of muscle.

Disturbance at the neuromuscular junction is exemplified by myasthenia gravis. The pattern of loss of volitional discharge, the recovery response to rest and antimyasthenia medications is similar in both types of muscle. Differences in pharmacologic sensitivity of ocular and peripheral muscle are, however, well known, as are certain anatomic modifications. The ocular electromyogram is perhaps the most sensitive test for myasthenia gravis currently available.

Supranuclear lesions are not easily recognized by electromyography. They are characterized by disturbances in reciprocity and pattern of firing but individual motor units are unaffected. These patterns are more readily recognized in ocular muscle and may lead to better topical diagnoses.

The preceding summary emphasizes the essential similarity of electric behavior of peripheral and extraocular muscle and should help to dispel the widespread notion that the two types of muscle are fundamentally different.

Quantitative aspects

Two approaches to electronic analysis of extraocular muscle potentials have been utilized in this study. The first is concerned with integration of activity; the second consists of the differentiation of potentials. Each will be discussed separately. They constitute the calculus of muscle activity.

A. Integration techniques

Integration consists of the summing up of the energy under the discharge curve of the action potentials. The integral is a function of both amplitude and frequency and electronic circuitry must take proper cognizance of both. Many types of integrating circuits have been employed in neurophysiology and, doubtless, many more will be developed.[3] The following account describes those with which we have had experience.

1. The electric data can be fed into a capacitor which accumulates the energy and discharges it in any of several ways.

a. The circuit can be designed so that the capacitor containing the stored data is rhythmically discharged at selected time intervals. The integral is expressed as an amplitude variation. This is the type previously reported by me.[6] The complete circuit diagrams are presented (figs. 5, 6, 7, 8, and 9). It is a composite instrument combining

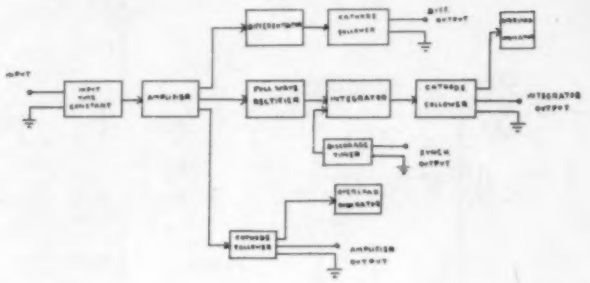


Fig. 5 (Breinin). Block diagram of Integrator-differentiator.

in one chassis both integrator and differentiator units plus certain accessories and is commercially available.*

* The instrument was designed to our specifications by Mr. George Katz, consulting engineer, and is sold by the Process and Instruments Co., Brooklyn, New York. It has been modified by our engineer, Mr. George Thomas. The changes in Figure 9 are not incorporated in the commercial instrument.

The block diagram shows that the signal from the electromyogram preamplifiers feeds a variable gain amplifier through selectable input time constants: D.C., 0.1 sec., 0.01 sec., 0.001 sec. This corresponds to a low frequency cut off (at the −3DB point, that is, where the amplitude of the signal is 50 percent of the midband amplitude) of approximately 1.6 cps, 16 cps, and 160 cps.

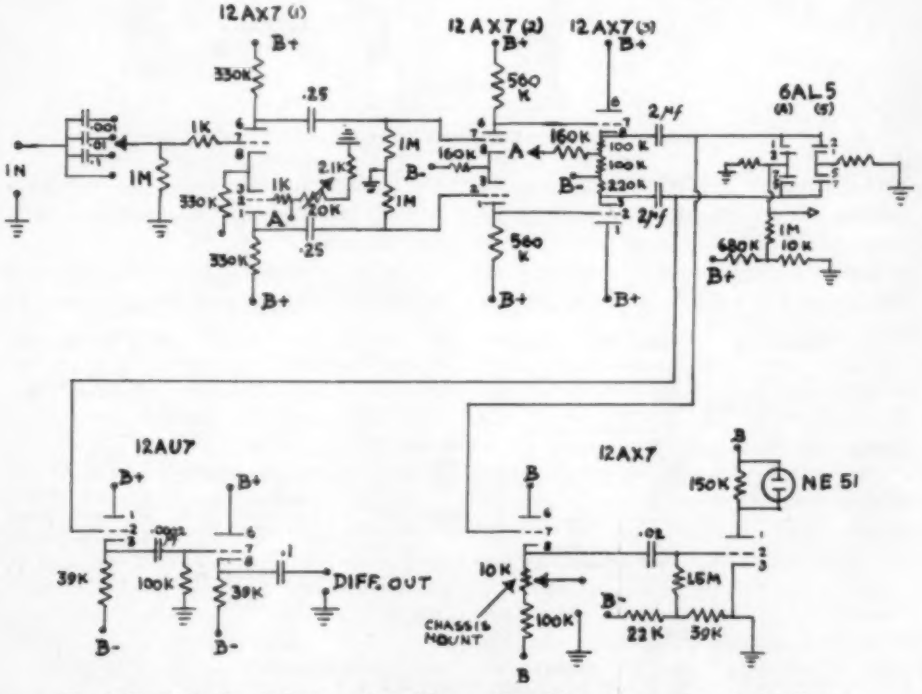


Fig. 6 (Breinin). Circuit diagram of amplifier, rectifier, differentiator, and overload indicator.

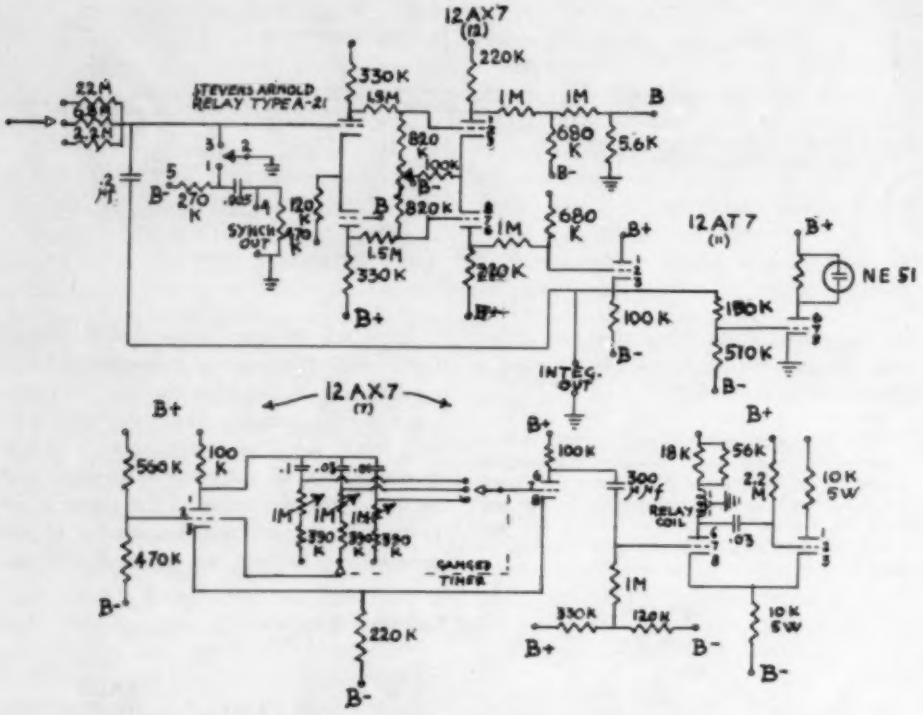


Fig. 7 (Breinin). Circuit diagram of timer, integrator, and overload indicator.

The amplifier consists of two cascaded, capacity coupled stages and a cathode follower output. The first stage serves mainly to convert a single ended to a balanced signal. This is followed by a balanced amplifier. The cathode follower drives the full wave rectifier, differentiator, and amplifier overload indicator.

The amplified signal is fed to a full wave rectifier which converts the potentials to

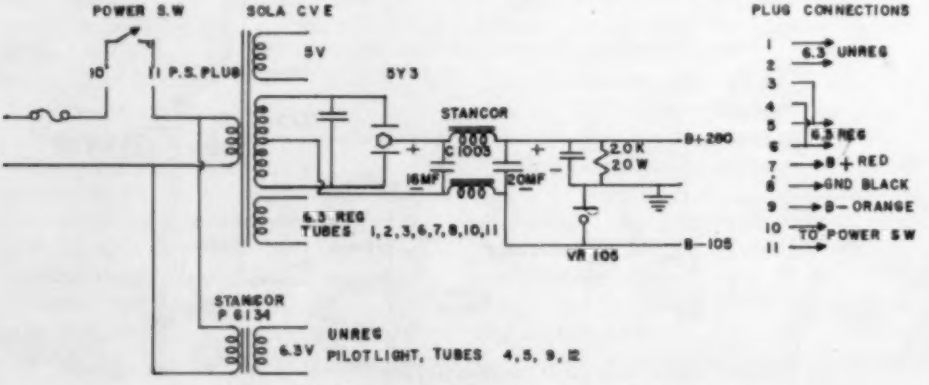


Fig. 8 (Breinin). Circuit diagram of power supply and plug.

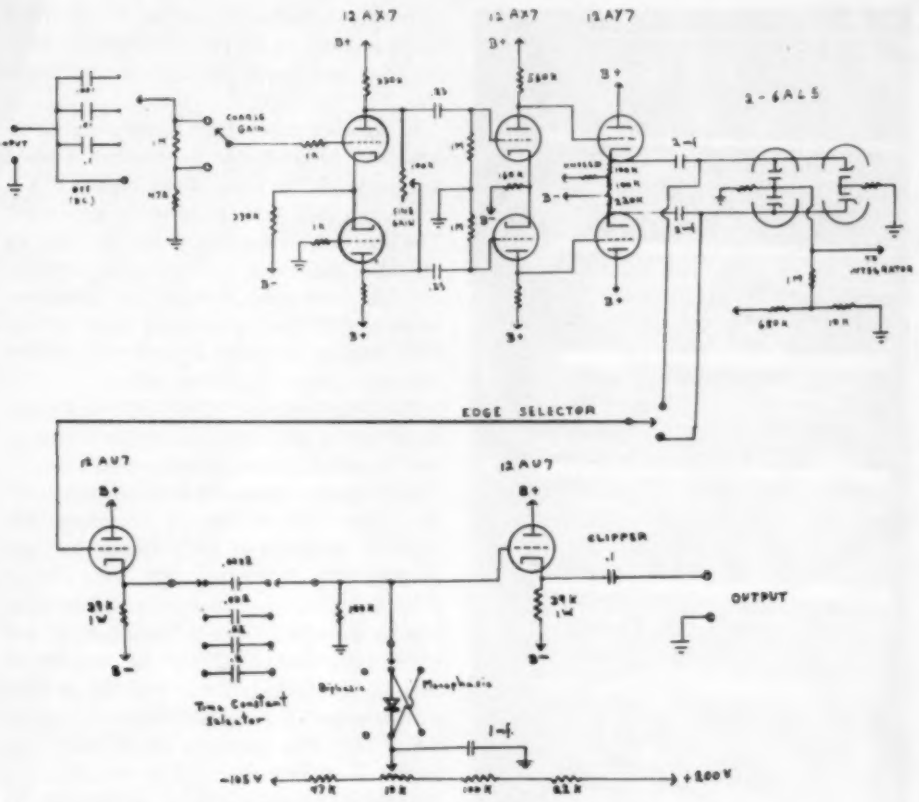


Fig. 9 (Breinin). Circuit diagram of modified integrator-differentiator.

D.C. pulses. These are stored in the Miller type integrator which consists of an RC system with selectable time constants and an amplifier to increase linearity. The output has a neon overload indicator to signal amplifier saturation. The capacitor is periodically discharged at one, three, or 10 times per second by a millisecond relay. A multivibrator supplies the pulses to the relay driver. A zeroing adjustment is incorporated to zero the output D.C. level. This is an important point for proper operation and the integrator output should be direct coupled to the y axis of the oscilloscope.

At the selected times the integrator discharges with a return to zero (ground) amplitude. The amplitude rise in the integrator is proportional to the integral with sufficient linearity for most purposes. The height of the discharge is the desired measurement. The process then repeats itself. This technique requires comparison measurements with a straight edge of the height of the repetitive discharges. A relative measurement of energy is thus available without reference to co-ordinates. An absolute measurement can be obtained by calibrating the output with a known input. A certain amount of noise will always be integrated hence a measurable baseline level is encountered without a signal but since noise is a random event it does not alter the relative measurements.

The integrator trace is simultaneously dis-

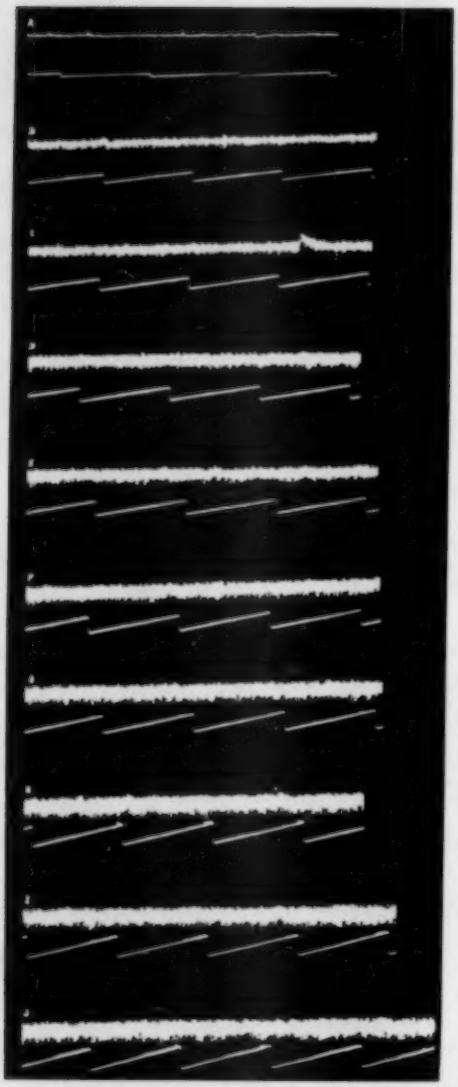


Fig. 10 (Breinin). Electromyogram of lateral rectus in 10 degree jumps (adduction to abduction). (Integrator lower trace, —50 to +40 degrees.)

played on a second oscilloscope beam hence its relation to the electromyogram is immediately obvious. This is a great advantage. Illustrative examples are presented in Figures 10, 11, 12, 13, and 14.

The system has a conservatively rated frequency response of five to 1,000 cycles which is adequate for muscle potentials. Both amplifier and synch outputs are also available.

b. Another method of integration (Bigland and Lippold[4]) is to establish a fixed amplitude charge level of the capacitor. On achieving this level a pulse is generated. The integral is expressed by the number of pulses generated (a frequency display). A higher integral charges the integrator more rapidly thus generating more pulses. This usually requires an external counter although a penwriter may suffice.

2. A third method (Drohocki[3]) is to full-wave rectify the signal and apply the envelope to an amplitude sensitive multivibrator. The integral is expressed as a frequency output. This must be counted externally. Capacitive integration is avoided by this method.

3. A fourth species of integrator is actually an average amplitude indicator. It was devised by Inman et al.[5] for studies on peripheral muscle and was recently modified by Momosse[7] for ocular electromyography (Fig. 15). The circuit is simple and quite useful for comparison of activity but has drawbacks for mensuration. It may be described as a continuous integrator. The block diagram shows that the signal is fed to an input amplifier which has a variable gain control. This permits attenuation of excessive signals. It also allows setting any signal to a desired output level and with a pulse source of known amplitude and repetition rate may be used to calibrate the output in a product of amplitude X frequency. The amplified signal is full wave rectified into D.C. pulses.

The integrator stores the pulses in a capacitor which is continuously discharged through a resistor. This allows the integrated output to follow the activity of the input signals depending on the time constant. The output level increases with increasing activity and decreases with decreasing activity. The time constant is selected to govern the

-50°	1.6	-40°	3.4	-30°	4.4	-20°	4.7
	1.6		3.7		4.2		4.6
	1.6		3.5		4.4		4.8
	1.8		3.4		4.6		4.8
-10°	5.1	0°	5.6	10°	6.0	20°	7.0
	5.0		5.4		6.2		7.1
	4.8		5.4		6.4		7.1
	5.0		5.4		6.4		7.0
30°	7.5	40°	7.6	AVERAGES:		-50°	1.7
	7.4		8.0			-40°	3.5
	7.3		7.8			-30°	4.4
	7.2		7.6			-20°	4.7
						-10°	4.9
						0°	5.5
						10°	6.3
						20°	7.1
						30°	7.4
						40°	7.8

Fig. 11 (Breinin). Integrator measurements in mm. (—50 to +40 degrees).

rate of decay for the type of activity being integrated. With R = 470 K, C = 0.47 uf, T = 0.22 second. This value is not critical. A choice of time constants can be readily incorporated in the circuit. The rectifier integrator combination is arranged for balanced operation of the output amplifier for increased stability. The output amplifier serves to amplify the integrated level and to increase the power. It is adequate to operate a penwriter.

The deficiencies of this system are that calibration co-ordinates are required and any D.C. drift will introduce errors. It should be emphasized that the circuit is continuously discharging and records average amplitude rather than true integral. Illustrative examples are presented in Figures 16 and 17.

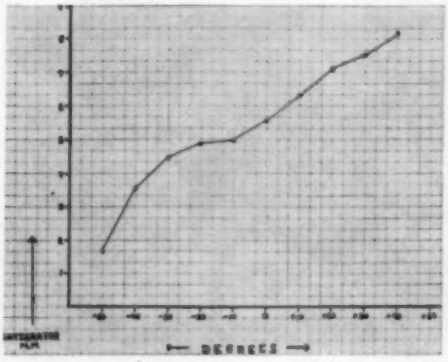


Fig. 12 (Breinin). Graph of integrator measurements of lateral rectus (—50 to +40 degrees).

B. Differentiation

A major problem in evaluating the ocular electromyogram arises from its high frequency and density. The analysis of frequency by inspection methods is tedious and difficult especially when units overlap—a common occurrence. The average electro-

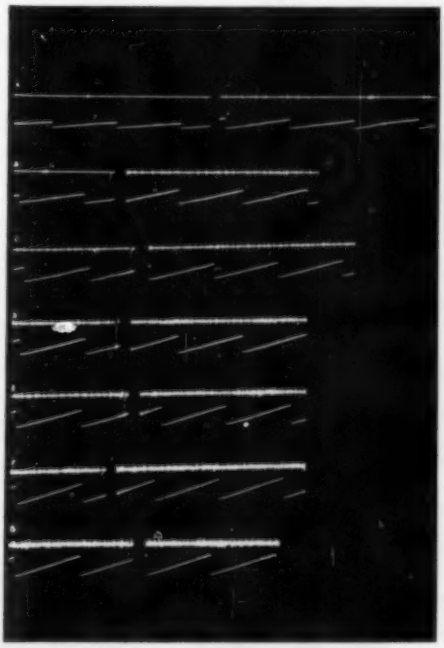


Fig. 13 (Breinin). Electromyogram of inferior oblique in 10 jumps (—40 to +30 degrees-depression to elevation). (Integrator lower trace.)

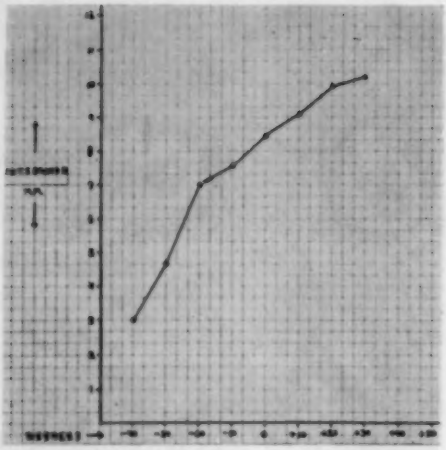


Fig. 14 (Breinin). Graph of integrator measurements of inferior oblique (−40 to +30 degrees).

myogram of ocular muscles reveals many units with humps and buttons due to this overlap. Fast sweep speeds do not always resolve the activity.

In an effort to get a truer and more automatic frequency analysis electronic differentiation of the wave form has been used. The input signal is amplified and fed to a cathode follower which drives the R-C differentiating network (fig. 9). A choice of time constants permits differentiation of signals of markedly different frequency. For electromyography a time constant of 20 μ sec. permits passage of signals or components in the neighborhood of 5,000 cycles or higher. Lower frequencies are discriminated against. By switching in longer time constants one may differentiate slower phenomena such as the electroretinogram. The output of the differentiator is passed through a cathode follower and is displayed on a second beam of the oscilloscope simultaneously with the signal. It is also fed into the computing system.

The differentiating circuit records a change of slope in the signal by a spike. A square wave elicits a spike at both the rise and fall, the points of inflection where activity is changing (fig. 18). The spikes are of opposite polarity and are very short duration. The faster the slope change (rise or fall time) the larger the differential spike. The amplitude of the differential will also follow that of the signal. In the case of a sine wave the differential is another sine wave but with

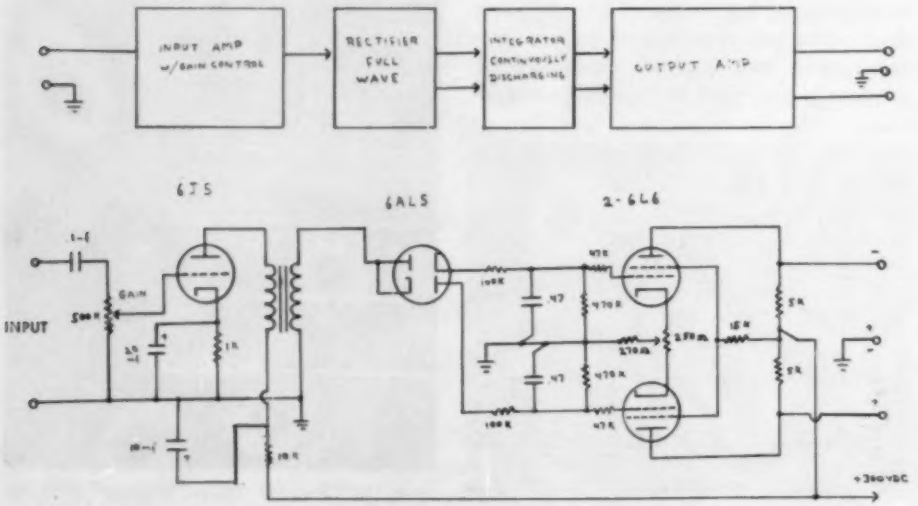


Fig. 15 (Breinin). Block and circuit diagram of modified Inman-Momosse continuous integrator.

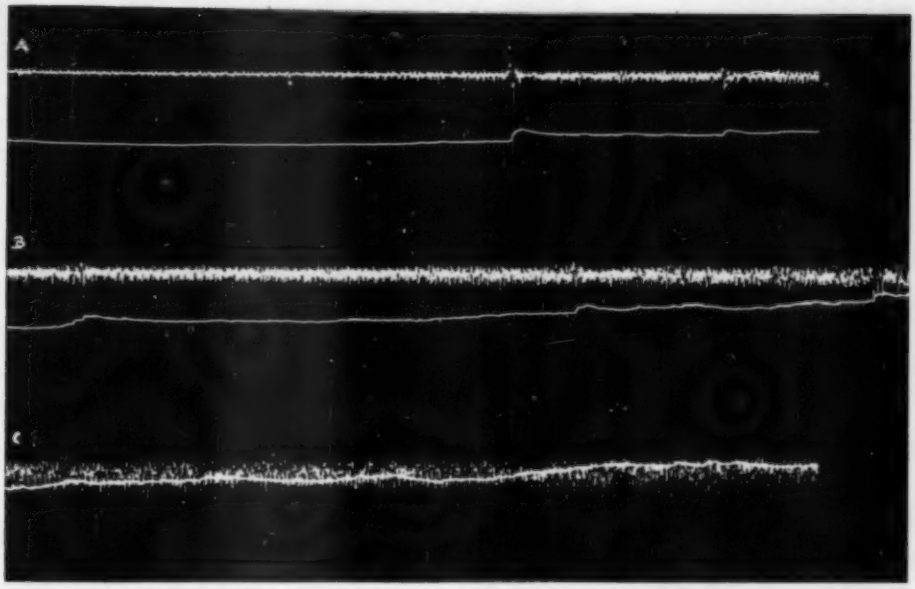


Fig. 16 (Breinin). Electromyogram of inferior oblique in gaze down to up. Continuous integrator—lower trace.

a 90-degree phase shift (cosine). A diphasic motor unit produces a diphasic differential spike; a monophasic units produces a monophasic differential spike. There is thus a 1:1 relation between a unit and its differential. However, each overlapping unit appearing as a button or hump on the signal is represented in the differential as a separate spike (figs. 19 and 20). Thus the obscure frequency components of the signal are trans-

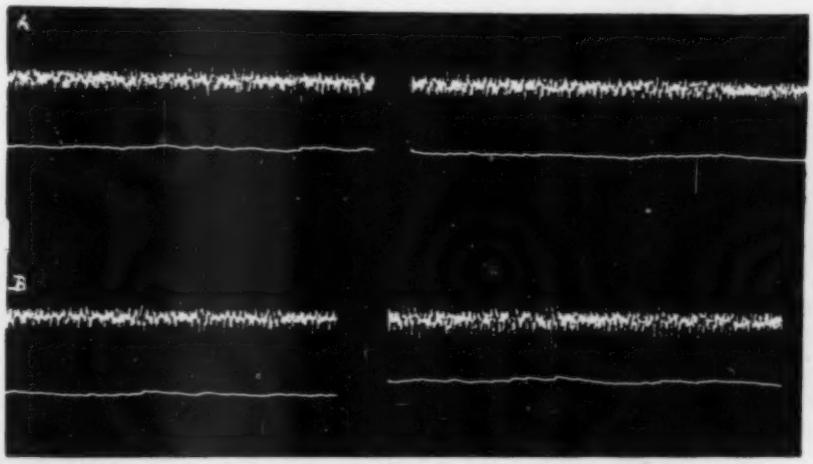
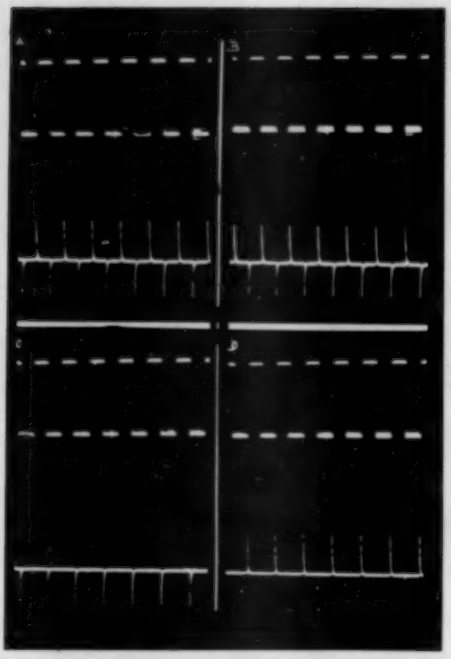


Fig. 17 (Breinin). Electromyogram of inferior oblique. Continuous integrator below. (A) Abduction-adduction: no change. (B) Primary position—elevation in adduction (increased).



lated into spikes which can be counted by inspection or, better, by electronic counters.

It has not proven possible to electronically analyze a signal containing many units in terms of frequencies of the individual units, even with a spectrum analyzer. All the pulses are counted together. This can be accomplished to a limited extent, however, by amplitude discrimination where amplitude differences exist.

The total pulse count appears to be a useful index to muscle activity. Increase of frequency and recruitment are basic mechanisms for augmentation of activity. Of course, amplitude variations are ignored by this system so that it does not truly integrate.

The differentiator base line is regular and

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Fig. 18 (Breinin). Differentiation of square wave. (A) Leading edge—positive. (B) Leading edge—negative. (C) Negative polarity. (D) Positive polarity.

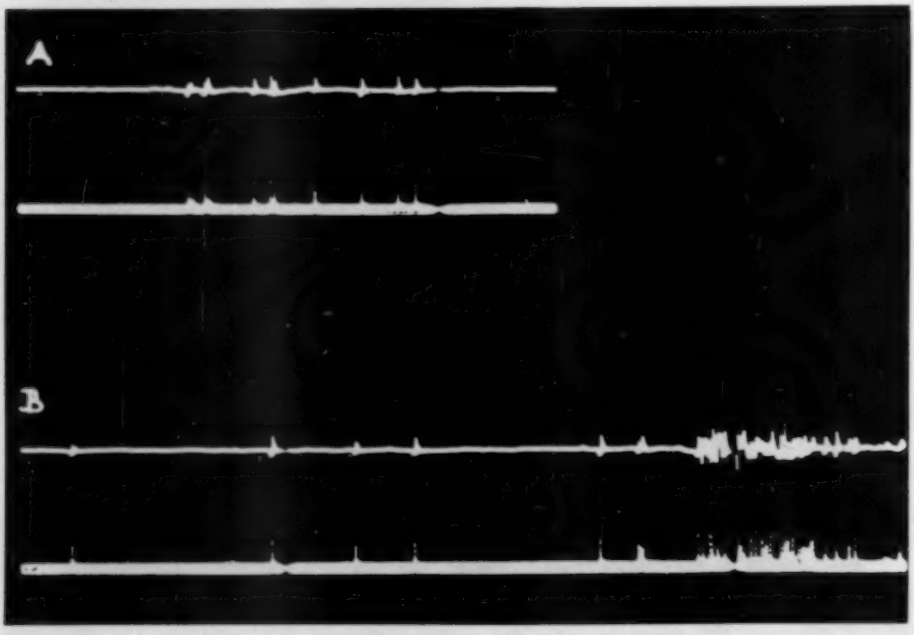

Fig. 19 (Breinin). Electromyogram of inferior oblique and differential (positive polarity). (A and B) Upper trace—signal; lower trace—differential.

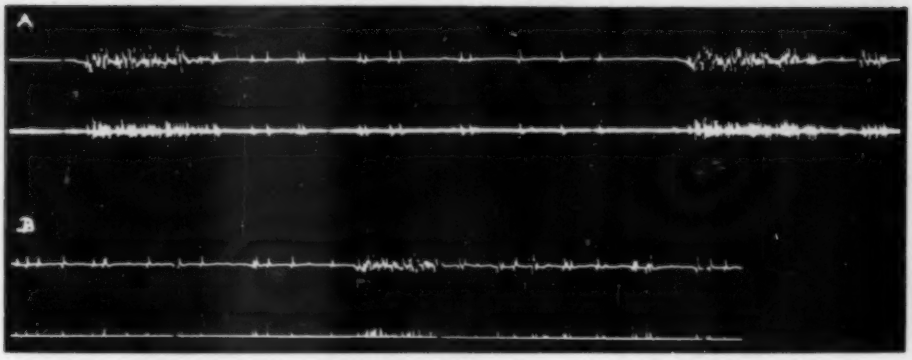

Fig. 20 (Breinin). Electromyogram of inferior oblique and differential. (A) Biphasic. (B) Positive polarity. Upper trace—signal; lower trace—differential.

devoid of the fluctuations of the signal. Movement artefacts are not reproduced. This allows more uniform counting rates than are obtained from the signal itself. Furthermore, the inherently high frequency motor units are neatly sorted out from 60-cycle activity and other slow waves (figs. 21 and 22). This permits one to extract the essential intelligence from signals that are obscured by interference. It is obvious that the differentiated signal has more abundant spikes than the signal itself (fig. 23). Even the resting baseline may exhibit a very "hairy" or "grassy" trace due to differentiation of noise. Controls are provided to attenuate such noise leaving only the signal.

Circuitry has been provided to select the leading or trailing edge of the signal as positive or negative. Furthermore, by means of a diode either positive or negative output polarity can be selected thus removing the positive or negative half of the differential (fig. 18). This reduces the total amount of data but still provides the essential information. (The counters operate on only one polarity.) It also helps in the visual interpretation of the trace. A clipper is provided to allow removal of baseline noise or to

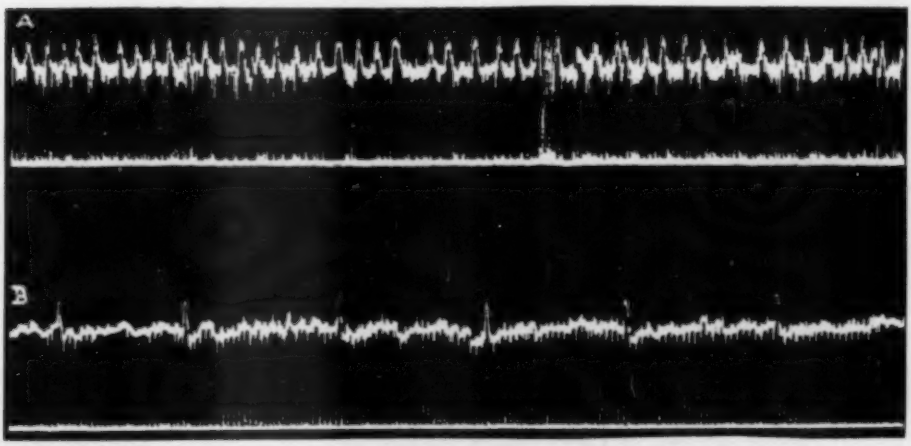

Fig. 21 (Breinin). Electromyogram of lateral rectus and differential (positive polarity). (A and B) Upper trace—signal with interference; lower trace—differential.

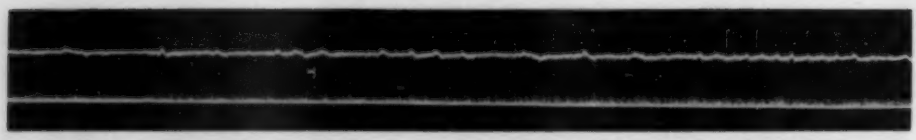

Fig. 22 (Breinin). Electromyogram of lateral rectus and differential (positive polarity). Upper trace—signal with motor units and slow waves. Lower trace—differential.

permit a count of spikes at selected amplitude levels.

Digitial recording

The counting of the pulses is accomplished by an electronic counter (Hewlett-Packard HP-522B) which has a response above 100 kc. The count may be obtained over selected intervals; one and 10 seconds are useful. In addition, a triggered or manual gate enables one to count a specific event, for example, a nystagmus burst. The total may be hand copied from the decade scales or may be printed automatically on paper by means of the automatic printing unit manufactured by the same company. These data cannot be displayed simultaneously with the signal but can be handled in standard statistical fashion (tables 1 and 2; figs. 26 and 27).

Analogue recording

The printing unit provides an analogue output which is proportional to the number of counts. This can be displayed on either a potentiometric or galvanometric recorder and presents a graphical record of the data (fig. 28).

The recording process, then, consists of photography of the oscilloscopic presentation of the signal and its differential, the digital printing of the differential count and its analogue recording. The data can also be simultaneously taped on the multichannel FM tape recorder (Ampex FR-1100) for repetitive study.

It is proper to note that the instrumentation necessary for all these steps produces a host of problems of maintenance. The services of an engineer are most helpful.

Experimental observations

The integrator and graphs reveal variations from linearity in the first phases of duction movement when the muscle is out

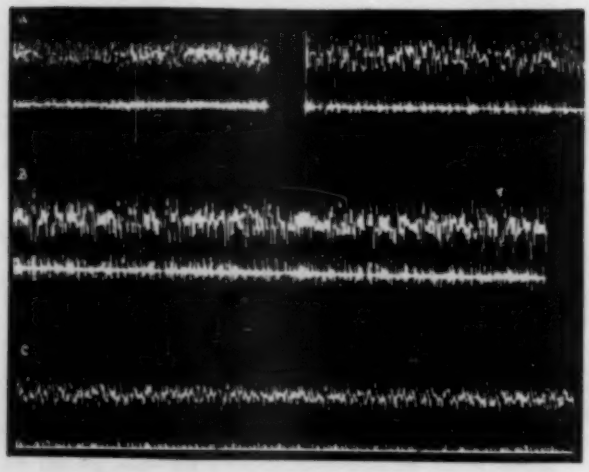

Fig. 23 (Breinin). Electromyogram of inferior oblique and differential. (A) Signal above, differential below (biphasic). Left, 10 cm./sec.; right, 25 cm./sec. (B) Same at 25 cm./sec. (C) Positive polarity of differential.

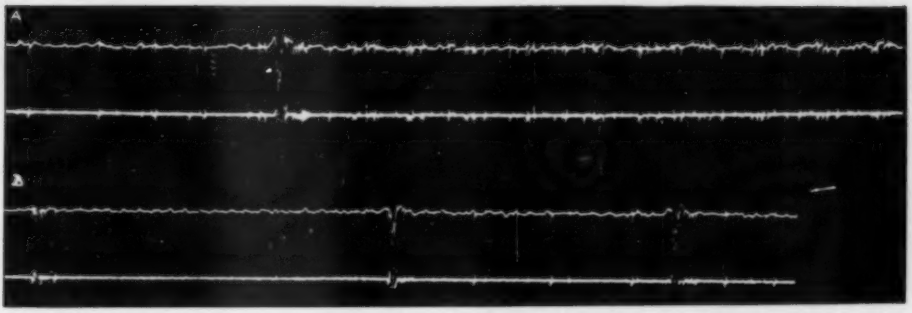

Fig. 24 (Breinin). Electromyogram of lateral rectus in nystagmus and differential. (A and B) Upper trace—signal; lower trace—differential (biphasic).

of its field of action. As the firing increases the linearity improves in both the lateral rectus in horizontal excursion and the inferior oblique in vertical excursion. The examples illustrate the value of integrating techniques in kinesiologic studies.

The use of differentiation resolves the motor units of the signal into a population of pulses. Nystagmus potentials consisting of many overlapping units and envelopes are represented in the differential by discrete pulses (figs. 24 and 25). These can be counted by inspection of the film or by use of the electronic counter.

The pulse counts of individual extraocular muscles in a given position of fixation are usually quite consistent over periods of several minutes. In Tables 1 and 2, 15 one-second counts showed some variation associated with small fixational irregularities and occasional small nystagmus bursts. The larger the pulse count and the longer the recording time the more consistent are the results (tables 3 and 4). The same count was obtained in the lateral rectus in gaze down and gaze up. This consistency was also demonstrated by the almost identical counts for distance and near fixation in the axis of the eye (table 5). These data corroborate studies with integration on vergence innervation.

However, the pulse count in repetition of different electrode positions may change considerably (table 6). This is a limiting factor in the application of the technique to a moving eye. With care in electrode positioning, nevertheless, it has been possible to get reproducible results. Further experience should clarify the reliability of pulse count-

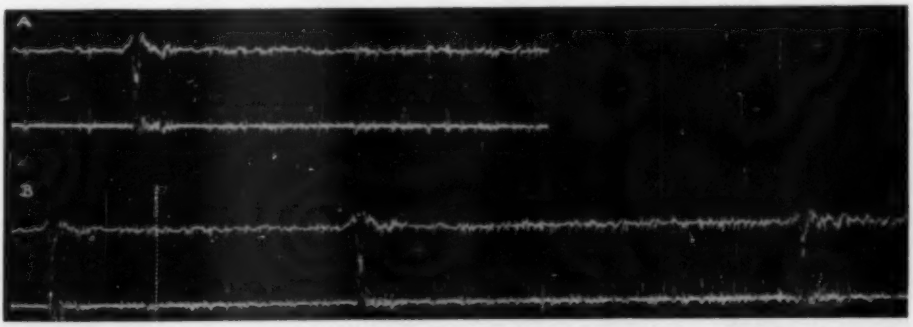

Fig. 25 (Breinin). Electromyogram of lateral rectus in nystagmus and differential. (A and B) Upper trace—signal; lower trace—differential (biphasic).

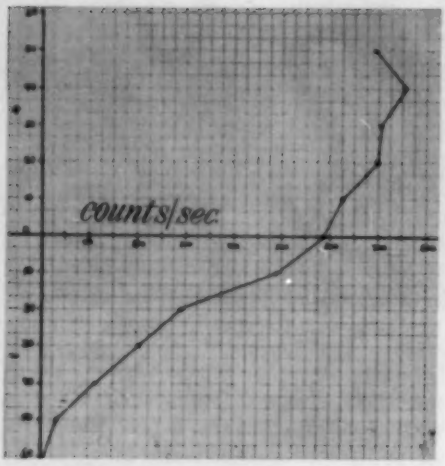

Fig. 26 (Breinin). Graph—left inferior oblique—frequency in movement: down to up.

ing as an index to activity. The count paralleled the movement in a case of aberrant 3rd nerve regeneration (table 7).

The relation of pulse count to movement is demonstrated in Figures 26 and 27. Good linearity is present over a large part of the excursion. Both muscles showed a fall of activity in the extreme field of action. This appeared to be due to electrode displacement rather than a true decrease of firing.

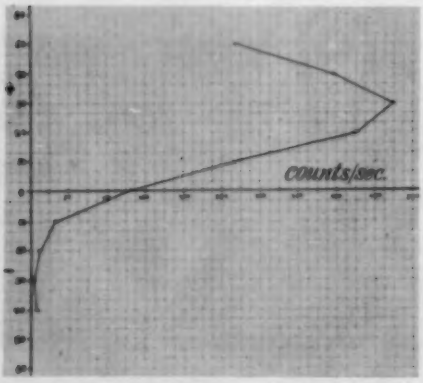

Fig. 27 (Breinin). Graph—right lateral rectus—frequency in movement: adduction to abduction.

TABLE 1

FREQUENCY COUNT OF RIGHT LATERAL RECTUS IN PRIMARY POSITION

Patient A. G., fixating O.D.

One-Second Count	Deviation from Mean
58	0.0
48	10.0
55	3.0
65	7.0
56	− 2.0
64	6.0
56	− 2.0
56	− 2.0
58	0.0
51	− 7.0
51	− 7.0
86	28.0
65	7.0
55	− 3.0
59	1.0
68	10.0
72	14.0
79	21.0
55	− 3.0
61	3.0
49	− 9.0
41	−17.0
53	− 5.0
68	10.0
54	− 4.0
55	− 3.0
51	− 7.0
Total 1578	
Total # 27	
Mean 58	

TABLE 2

FREQUENCY COUNT OF LEFT INFERIOR OBLIQUE IN PRIMARY POSITION

Patient M. U.

One-Second Count	Deviation from Mean
254	+ 2
272	+20
297	+47
278	+26
258	+ 6
229	−22
212	−40
229	−22
246	− 6
264	+12
270	+18
256	+ 4
235	−17
241	−11
231	−20
Total 3792	
Total # 15	
Mean 252	

TABLE 3

FREQUENCY COUNT OF LEFT LATERAL RECTUS IN PRIMARY POSITION

Patient L. T.

Ten-Second Count	One-Second Count
10,000	1,043
9,931	1,029
10,101	1,030
10,047	1,074
10,003	1,023
	1,069
	1,071
	1,069
	1,041
	1,094
	1,073
	1,024
	1,020
	1,026
	1,018
Total 50,082	Total 15,704
Average 10,016 (10 sec.)	Average 1,047
Average 1,002 (1 sec.)	

In the field of action high pulse counts are encountered—often well over 1,000 per second. Care is necessary to adjust the differentiating amplitude level to avoid counting noise, which yields extremely high counts. The amplitude level is set so that a low count is obtained when the tested muscle is fully out of its field of action. The low counts at the onset of movement in Figure 27 were due to a very low amplitude level setting.

TABLE 4

FREQUENCY COUNT OF LEFT LATERAL RECTUS GAZING UP AND DOWN

Patient L. T., O.S., fixating; O.D., covered; one-second count

Gaze Up	Gaze Down
1,218	1,232
1,195	1,237
1,220	1,237
1,253	1,216
1,230	1,190
1,209	1,224
1,223	1,216
Total 8,548	Total 8,552
Average Per Sec. 1,221	Average Per Sec. 1,222

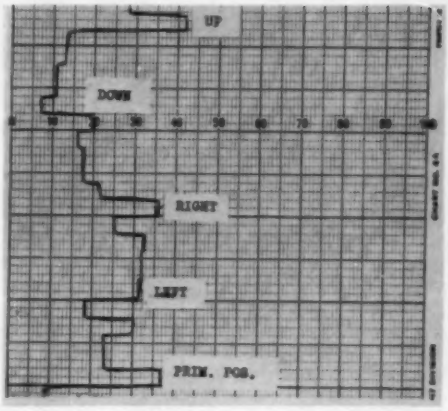

Fig. 28 (Breinin). Graph—analogue record, right inferior oblique.

DISCUSSION

Tension in the nerve muscle preparation is a function of frequency. In intact human muscle Lippold[12] has shown that a linear relation exists between the integrated electromyogram (by planimetry) and tension in voluntary isometric contraction. No proportion was found between mechanical and electric responses of a single motor unit but when the summated effect of a large number of units was recorded the variation statistically cancelled out. He assumed that recruitment must be random. Kuboki[13,14] has reported the varying time series discharges of single units in extraocular muscle, their irregularities at certain frequencies, and the lack of relationship existing between the activity of certain units and the ocular movement. In other instances he noted that units increased firing in accordance with the movement. I have observed similar phenomena. It would appear unwise to expect the correlation of single unit discharges with muscle tension but the over-all sampling of the muscle does reflect a significant relationship of frequency to movement (figs. 26 and 27).

Bigland and Lippold[4] found a direct proportion between integrated electric activity and tension during constant velocity of

TABLE 5

FREQUENCY COUNT OF LEFT LATERAL RECTUS AT DISTANT AND NEAR

Patient L. T., approximation in axis, O.S., one-second count

Distant		Near	
	959		956
	1,015		925
	969		947
	908		982
	934		911
	936		986
	989		1,029
	977		909
	1,021		939
	987		1,006
Total	9,695	Total	9,590
Average Per Sec.	969	Average Per Sec.	959

shortening or lengthening, and a linear relation between tension and the number and frequency of units. Inman et al.[5] found a linear relation between electric integral and tension in isometric contraction with monopolar, surface and coaxial electrodes but no quantitative relation when the muscle was permitted to change in length.

These differences may be related to the manner in which the studies were performed. Momosse discusses some of these points in Japanese.[7]

It is of interest that all types of electrodes gave comparable results but it seems reasonable that the larger the pick up area the more significant would be the data. Very fine electrodes, designed to sample single units, could not accurately portray the over-all electrical pattern.

The electromyogram can be passed through frequency and amplitude discriminating circuits yielding data of varied nature. These data can be reintegrated to give information concerning specific electric activities of the signal. The research possibilities inherent in these approaches are very broad and justify intensive investigation of integration-differentiation techniques.

TABLE 7

FREQUENCY COUNT OF RIGHT INFERIOR OBLIQUE

Down, up, right, left, primary position

Position	Six-Second Count	One-Second Count
Down	30	5
Up	1,281	213
Right	728	121
Left	1,235	205
Primary	440	73

SUMMARY

1. A comparison of the ocular and peripheral skeletal muscle electromyogram in health and disease reveals a fundamental similarity of the two types of muscle, although differences do exist.

2. Integrating techniques permit detailed kinesiologic studies of innervation versus movement. Various integrator circuits are described.

3. Differentiating techniques convert the signal to pulses. These yield a more accurate picture of the frequency components of the signal. The data can be subjected to electronic counting and graphing. Differential circuitry is described.

TABLE 6

FREQUENCY COUNT OF RIGHT LATERAL RECTUS

Patient D. L., right, primary position, left

Position	1. Six-Second Total	1. Average Per Second	2. Six-Second Total	2. Average Per Second	3. Six-Second Total	3. Average Per Second
Right	1,666	277	7,382	1,230	7,241	1,206
Primary	1,044	174	3,647	607	2,552	506
Left	53	8	127	21	40	7

4. The combination of electronic integrating and differentiating techniques opens new avenues of investigation of electric activity in muscle.

ACKOWLEDGMENT

Appreciation is extended to Mr. George Thomas, E.E., for valuable advice, to Mr. Walter Lentschner, photographer, and to Mr. Freeman York who assisted in these studies.

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DISCUSSION

LIVINGSTON: I should like to ask Dr. Breinin to what degree he has observed co-contraction of antagonists in his analysis of extraocular muscle activities? In the waking individual are antagonists showing a good deal of activity of most positions of the eye? A related question concerns whether the degree of activity—the total amount of activity in all of the muscles—may reflect the degree of alertness or somnolence and thereby provide a physiologic reflection of levels of brain stem activity in relation to arousal?

FUORTES: Are there any questions or comments? If there are not, I should like Dr. Breinin to reply to this question.

BREININ: Simultaneous increase of activity of antagonists is not a normal phenomenon in the extraocular muscles, although it can occur. There is a constant tonic level of activity of all the muscles in the primary position. In vertical movements the horizontal muscles tend to maintain the same level as do the vertical muscles in horizontal movements. However, in some pathologic conditions the activity of antagonists increases or decreases together. In studies of asymmetric convergence, my experience is that if you approximate an object in the axis of the fixating eye, the muscles of the fixating globe show no alteration of their horizontal innervation as you come in until suddenly simultaneous increase of antagonistic activity may occur. Thus, co-contraction occasionally occurs in normal individuals as a type of defense mechanism. Movement toward the eye with a tonometer, for example, may result in co-contraction.

The second question concerned arousal and drowsiness. When a person falls asleep, ocular muscle activity decreases sharply and then little irregular bursts, alternating with periods of silence, may occur. On arousal, the firing starts spiking. If you give the subject an anesthetic, the muscles become completely silent; as he comes out of the anesthesia, they fire again. I think that this activity is correlated with arousal mechanisms, probably through the reticular formation. It would be interesting to study the correlations between cortical activity and the ocular electromyogram. How these influences operate in waking persons, I don't know. There are differences in level in different people, but I've seen some lethargic individuals who had very good activity, and some alert individuals who did not.

FUORTES: Thank you very much, Dr. Breinin. There is one more question. Dr. Hubel.

HUBEL: I would like to ask in what way does simply closing the eyes differ from sleeping and thus closing the eyes?

FUORTES: Dr. Breinin.

BREININ: There is no correlation between sleeping and closing the eyes. Behind the closed lids the muscles fire perfectly well. The only typical occurrence may be Bell's phenomenon, revealed by increase of activity of the inferior oblique. As a matter of fact, this was one of the ways in which we diagnosed a supranuclear lesion. In a patient who had a cerebral accident, we found that behind closed lids the eyes deviated to the left. When the person opened the eyes, deviation was observed to the right. This was an expression of the entity that Cogan has described as spasticity of conjugate gaze.

FUORTES: Thank you very much, Dr. Breinin. We shall proceed now to the next and last paper, and this is by Dr. George Goodman and Dr. Ralph Gunkel, the title being "Familial electroretinographic and adaptometric studies in retinitis pigmentosa." Dr. Goodman.

FAMILIAL ELECTRORETINOGRAPHIC AND ADAPTOMETRIC STUDIES IN RETINITIS PIGMENTOSA*

GEORGE GOODMAN, M.D., AND RALPH D. GUNKEL, O.D.
Bethesda, Maryland

Since the original report by Karpe[1] on the absence of the electroretinogram in primary retinitis pigmentosa, this observation has been corroborated by several investigators in over 150 reported cases.[2-9] It has become generally accepted that the electroretinogram in this condition is usually extinguished, or manifest only as a small negative response.[9-11]

Electroretinography has been routinely used at the National Institutes of Health for the past two years in the study of retinal anomalies and diseases. With the intense light stimuli used in this laboratory, several cases of primary retinitis pigmentosa have demonstrated small but well-defined responses. These findings made it advisable to study the children of patients with retinitis pigmentosa in order to determine the nature of the electroretinographic response in early cases.

The electroretinogram was investigated in 13 members of two families with retinitis pigmentosa of dominant inheritance. The subjects ranged in age from one to 64 years. Dark-adaptation curves, perimetric light sense thresholds (retinal profiles), and visual

* From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

fields were also studied. Techniques were utilized in the electroretinogram and subjective tests which made possible a separate evaluation of the photopic (cone) and scotopic (rod) response. The clinical, physiologic, and genetic implications of the findings are discussed.

Methods

Each subject underwent a complete ophthalmologic examination and electroretinographic testing. Visual fields, adaptometry, and perimetric light sense studies were carried out in all those above five years of age. The five youngest children in the B family, all below the age of six years, were put to sleep with barbiturates in order to perform the electroretinogram and fundus examinations.

1. *Color vision.* The Hardy-Rand-Rittler pseudoisochromatic plates and the Farnsworth D-15 Panel were used for color vision testing.

2. *Visual fields.* Central visual fields were examined on a tangent screen at one meter. Peripheral visual fields were tested on the Goldmann projection perimeter. In most subjects a preliminary peripheral field was obtained with the largest target (64 mm.^{2}) at full intensity. The 1/4 mm.^{2} stimulus was then used at four intensities. This target is designated by Goldmann as size 1, and the four intensities as 1 (0.03), 2 (0.09), 3 (0.31), and 4 (full intensity). The test objects are referred to in illustrations and text using the Goldmann symbols, with target size as the numerator, and stimulus intensity as the denominator of a ratio, for example, 1/4 mm.^{2} target, intensity 0.31, is ⅓. The normal isopter with target 1/4 is sketched in Figure 4; this represents the average isopter of 30 young persons tested by Goldman and is essentially a full peripheral field.

3. *Adaptometry.* Dark adaptation curves were obtained with the Goldmann-Weekers adaptometer after a 10-minute preadaptation to a 310 millilambert light surface in the integrating sphere. The preadaptation light was measured with a Macbeth illuminometer. The test patch was 11 degrees in diameter with its center 11 degrees below a small red fixation light. Thresholds of apperance and disappearance of the light stimuli were measured by means of an automatic intensity control attachment to the adaptometer which was operated by the patient. Details of this apparatus, and the testing procedure used in this laboratory, are described by Gunkel and Bornschein.[12]

4. *Perimetric light sense studies (retinal profiles).* These studies were suggested by the work of Sloan,[13] Mandelbaum,[14] and Zeavin and Wald.[15] The Goldmann-Weekers adaptometer was modified so that retinal profiles could be obtained with white, red, and blue stimuli at zero, two, five, 10, 20, 30, and 40 degrees from fixation in superior retina, along the vertical meridian. This was made possible by the use of a movable fixation light whose position could be changed by means of a dial on the outside of the adaptometer sphere without disturbing the patient. The light emerged from the drawn out end of a lucite rod as a fine point; a variable resistance permitted it to be kept just bright enough to allow adequate fixation. A slide was prepared for insertion in front of the light source, which contained three circular openings each subtending an angle of two degrees at the subject's eye. One aperture was left open for white light, the second contained a red filter, Wratten No. 29, and the third a blue filter, Wratten No. 47.

The threshold determinations began after completion of the dark-adaptation curve, which had been preceded by light adaptation, or after 15 minutes in darkness with no preliminary light adaptation. Results under both circumstances were identical. Testing proceeded from fixation toward the periphery on the right eye of each subject measuring achromatic thresholds. A retinal profile was completed separately for each color in the order white, blue, and red. Automatic recording by the patient was employed as in

the dark adaptation curves. At least four values were plotted for each point (two thresholds of appearance and two thresholds of disappearance) which required about one minute of continuous testing; this was followed by one minute of rest before moving to the next point. The entire procedure lasted about 45 minutes. Normal retinal profiles were established by testing employees, staff members, and hospital patients with no evidence of eye pathology.

Subjects B III 5 and B III 9, both five-year-olds, were too young to co-operate in the adaptometric studies, and did not tolerate the preadaptation light. An attempt was made to determine red, white, and blue thresholds after 20 minutes of dark adaptation with no preadaptation, using the 11 degree light stimulus, random fixation, and binocular reporting. Ten determinations of thresholds were made during a five-minute period and the results were checked in a second testing session. The measurements were consistent and reproducible for both subjects.

5. *Electroretinography.* The electroretinogram was recorded using a contact lens electrode, a reference electrode on the bridge of the nose, and a ground electrode on the forehead. The Burian-Allen speculum contact lens electrode was used in all adults, but the plastic speculum to which the contact lens is attached was found to be too large for the palpebral apertures of the four youngest children (B III 6, 7, 9, 10). For these subjects contact lenses were pressed from transparent methyl methacrylate (plexiglass) with the corneal part of a curvature radius smaller than that of the scleral part. The electrode was a short piece of $\frac{1}{16}$-inch silver rod screwed into the shell and polished on the inside. No speculum was necessary since the electroretinograms were recorded while the children were asleep and lid movements were minimal.

The electrodes were connected directly to the amplifying system of a direct-writing, eight-channel electroencephalograph (Grass, model IIID). The over-all frequency response was found to be constant from 0.5 to 80 cycles/sec. The electroencephalogram filter was used to obtain a steadier baseline. This increased the lower frequency limit to about two cycles/sec., which was still well below the fundamental frequency of the a-, b-, and x-waves examined in this study. The advantages of this Grass electroencephalograph for electroretinographic recordings were described by Armington.[16]

The Grass Photic Stimulator (model PS1) was the light source. This consists of a xenon discharge lamp with a flash of about 10 microseconds' duration, and variable intensity and frequency of discharge. The energy distribution of the lamp without filters is about 90 percent from 400 to 575 millimicrons, and then drops off to a low of about 70 percent at 675 millimicrons. The peak luminance, calculated on the basis of figures supplied by the manufacturer, is about 6×10^7 millilamberts at intensity setting No. 16. Intensities 12.5, 25, 50, and 100 in this study refer to settings 2, 4, 8, and 16 respectively on the Photic Stimulator when modified by a white plastic diffuser with a luminous transmission of about 42 percent.

The Photic Stimulator was set into a blackened cylinder which was equipped with a holder for filters. The front glass of the flash lamp was about 12 inches from the subject's eye. At this distance the diameter of the stimulus patch was about 30 degrees. The spectral composition of the light was altered by means of a Grass red filter (about 90-percent transmission beyond 650 mμ, and less than 10-percent transmission below 600 mμ) and Wratten blue filter No. 47B (maximum transmission at 430 mμ and less than 10 percent below 390 mμ or above 470 mμ.)

The subject's eye was dilated with 10-percent neosynephrine and 1.0-percent cyclogyl. Light-adapted tracings were obtained with a surround illuminance of about 12 foot-candles after previous exposure to an average room illumination of about 18 foot-

candles. Single flash stimuli were delivered at a rate of one per second with intensity 12.5 and 100; in some cases the response to 30 per second flickering stimuli was also recorded. After eight minutes of dark adaptation the responses to intensity 12.5 and 100 were again recorded, with intervals between flashes not less than 30 seconds. In two subjects (B III 9 and B III 10) this was followed by six minutes of preadaptation to a 1,000 millilambert light source subtending a visual angle of about 90 degrees. Stimulus 100 was then delivered after 20 seconds, 1, 2, 3, 4, 5, 8, 10, 13, 16, and 20 minutes of dark adaptation. The responses to red and blue stimuli were studied in four subjects (B II 2, B III 8, G IV 1, and G IV 2) after 10 minutes of dark adaptation without a preliminary light adaptation.

In the light-adapted state the subjects fixed on the center of the stimulus patch; in the dark-adapted state a fluorescent dot in the middle of the diffuser served as the fixation mark. In the sleeping children there was some tendency for the eyes to roll up, but scotch tape sufficed to hold the lids open and completely expose the pupil. Care was taken to center the stimulus patch over the eyes of subjects and to angle the flash lamp so that the front glass was perpendicular to their visual axes.

The following medications were used to induce sleep in the five youngest children: rectal pentothal (B III 6, 7, 9, 10), intramuscular nembutal and demerol (B III 8), and rectal pentothal and intravenous nembutal (B III 5). The possibility that the barbiturates might affect the electroretinogram was considered. Electroretinograms were done in three patients, including B III 8 of this study, under normal conditions, and during a barbiturate-induced sleep; the amplitude and shape of the waves were similar with and without medication. Furthermore, experimental studies in the cat have shown that unless massive doses of barbiturate are used, or large doses are administered intra-arterially, no effect on the electroretinogram can be demonstrated.[10a] In this regard it is important to note that two of the children in this study were so lightly sedated that they awoke during testing, and the other three were easily awakened at the termination of the examination. (B III 5 showed an excessive drowsiness for a 24-hour period after the test was over which the supervising anesthesiologist attributed to an idiosyncrasy to the barbiturate used.)

Case histories*

G Family

The pedigree of the G family is presented in Figure 1. The propositus G III 1 is a 32-year-old white man who was referred to the National Institutes of Health with a diagnosis of retinitis pigmentosa. He reported a history of night-blindness in three other siblings (G III 2, 4, and 7) as well as four antecedents in two previous generations (G II 2, 3, 5, and I 1). He was also certain that his nine-year-old daughter (G IV 2) was night-blind and had been so since early childhood, while the two other children (G IV 1 and 3) were said to be unaffected. His brother, G III 2, showed contracted side vision even as a child and is now considered legally blind. The older affected members are said to maintain fair vision during the day.

A full examination, including electroretinogram, visual fields, adaptometry, and perimetric light sense studies, was obtained from the propositus, two of his children (G IV 1 and 2), and a sister (G III 4). The remaining affected members all reside in southern Illinois and could not be studied.

G III 1

This 32-year-old television entertainer first became aware of night-blindness at about the age of seven years. He and other affected members of his family stated that their night-blindness had not changed in degree since they first noticed it in childhood. During the past five years, however, he noted a progressive loss of side vision in normal illumination. He also claimed poor color discrimination throughout his life, but he did not know if other members of the family were so affected.

Visual acuity. 20/40 uncorrected, O.U.; 20/20 corrected, O.U., with −0.5D. sph. ⊂ +1.25D. cyl. ax. 75°.

Color vision. Deuteranopia.

Anterior segments. Discrete centrally located posterior subcapsular opacities, O.U.

Fundi. The optic discs were normal. There was a thin ring of chorioretinal atrophy appearing like a halo around each disc. The vessels showed slight

* Patient G III 1 was referred by Dr. Lucian Bauman and patient B II 2 by Dr. Bernard Gurwin.

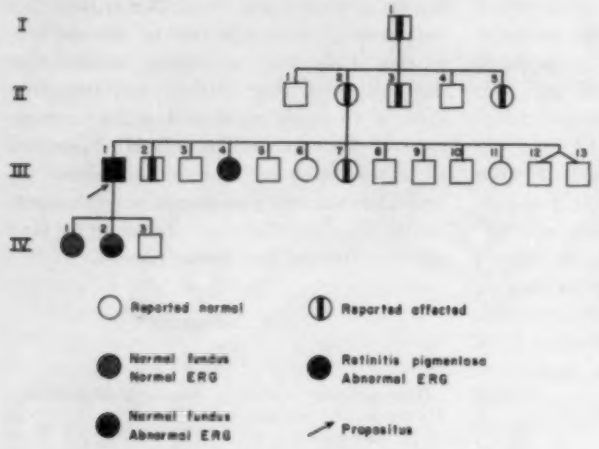

Fig. 1 (Goodman and Gunkel). Pedigree of the G family in which retinitis pigmentosa is inherited as an autosomal dominant trait. The ages of the subjects under study were: III 1—32 years; III 4—28 years; IV 1—11 years; and IV 2—nine years.

attenuation. Delicate spidery bone corpuscle pigmentation was irregularly dispersed in the preequatorial zone of both eyes.

Visual fields. The peripheral visual field, O.S., is depicted in Figure 3. With target 1/4 a ring scotoma was found with extension to the periphery, while dimmer stimuli revealed only a central island of vision. Similar changes were present, O.D. Central fields were 13 degrees with a 10-mm. white target at one meter, and four degrees with a 1.0 mm. target.

Dark-adaptation curve. The cone segment was normal, the "break" occurred at about 8.5 minutes, and the scotopic increment was reduced so that the threshold at 25 minutes was $10^{3.4}$ μμl—almost 1.5 log units above normal (fig. 5).

Retinal profiles. Only the white light stimuli were appreciated beyond 10 degrees, with a gap in the profile present between 10 and 40 degrees. Within the 10-degree field the foveal red thresholds were within normal limits; otherwise both blue and red thresholds were elevated, with the red thresholds showing less elevation above the norm than the blue (fig. 5).

Electroretinogram. The responses in both light- and dark-adapted states were similar and showed a negative and positive deflection which were barely discernible above baseline activity. (See Figure 9 for original records and Table 1 for amplitude measurements.)

G III 4

This 28-year-old housewife first became aware of night-blindness at about 10 years of age; she felt that it had remained stationary since then. Since the age of 16 years she has noted poor side vision

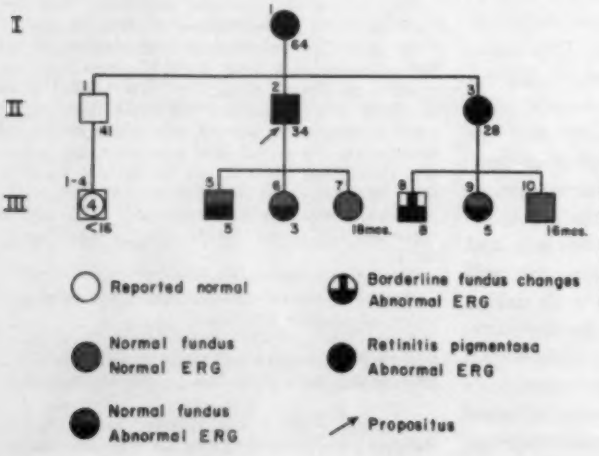

Fig. 2 (Goodman and Gunkel). Pedigree of the B family in which retinitis pigmentosa is inherited as an autosomal dominant trait. The number below each subject refers to age in years, unless otherwise indicated. The mother of I 1 may also have been affected.

TABLE 1

STUDIES OF THE B. FAMILY AND G. FAMILY

Subjects	Light Adapted		Dark Adapted	
	Negative Wave Amplitude (microvolts)	Positive Wave Amplitude (microvolts)	Negative Wave Amplitude (microvolts)	Positive Wave Amplitude (microvolts)
G. III 1	18	20	18	18
G. III 4	24	36	30	98
G. IV 2	48	190	172	252
G. IV 1	80	155	273	510
B. I 1	0	0	0	0
B. II 2	37	87	94	100
B. II 3	31	44	42	50
B. III 8	56	169	132	140
B. III 5	—	—	162	119
B. III 9	75	131	162	172
B. III 6	—	—	156	206
B. III 7	—	—	225	319
B. III 10	50	106	175	356

Amplitude of negative and positive waves in the electroretinographic response to intensity 100, light- and dark-adapted conditions, for members of B. and G. family. Original records are shown in Figures 9 and 10. The positive response was measured from the trough of the negative wave (a-wave) to the highest positive crest. In responses with two positive humps, therefore, only the maximal positivity was measured. The amplitude of the positive responses in clinical electroretinography of normal subjects under these conditions exceeds 75 μv for the light-adapted positive response, and 300 μv for the dark-adapted positive response.

during the day. She has three children, four years, two years, and eight months of age, who have not shown any difficulty in navigating under dim illumination.

Visual acuity. Uncorrected vision was 20/40, O.D., and 20/50, O.S. Corrected vision was 20/20, O.U., with −0.75D. sph. ⌒ +0.75D. cyl. ax. 125°, O.D.; −1.0D. sph. ⌒ +1D. cyl. ax. 75°, O.S.

Color vision. Normal.

Anterior segments. No abnormalities.

Fundi. Discs and maculas were normal. Retinal arterioles showed mild but definite attenuation. The major change throughout the retina was a patchy retinal depigmentation with baring of choroidal vessels, which appeared subnormal in caliber. There were two or three bone corpuscle pigment deposits detectable in each eye.

Visual fields. Central visual fields, O.U., were constricted to 20 degrees with a 10-mm. white target at one meter, and to eight degrees with a 1.0-mm. white target at one meter. Peripheral field testing was done on the right eye only. With the largest (64 mm.^{2}) target at full intensity a ring scotoma was disclosed in the superior field between 25 and 45 degrees. There was a central 15-degree field and a small remaining segment of intact field between 30 and 40 degrees in the inferior nasal quadrant with the 1/4 target.

Dark-adaptation curve. The cone segment was elevated slightly above normal, the "break" was delayed until 12 minutes, and the scotopic increment was reduced so that the threshold at 25 minutes was 10^{-5} $\mu\mu$l—over 1.5 log units above normal (fig. 5).

Retinal profiles. Thresholds were elevated at all points tested, with a marked increase beyond 10 degrees. The blue thresholds were relatively more abnormal than the red (fig. 5).

Electroretinogram. The light- and dark-adapted electroretinograms with stimulus 100 were small but well-defined responses which consisted of a negative wave preceding a positive deflection. The amplitude of these waves showed little change from light- to dark-adapted state, though there was some broadening of the dark-adapted positive response. Only a small positive deflection was elicited by the 12.5 stimulus when the patient was dark-adapted. (See Figure 9 for original records, and Table 1 for amplitude measurements.)

G IV 1

This 11-year-old schoolgirl had no ocular complaints.

Visual acuity. 20/20, O.U., uncorrected.

Color vision. Normal.

Anterior segments. Normal.

Fundi. No abnormalities, O.U.

Visual fields. Central visual fields were full with a 1.0-mm. white object at one meter. Peripheral visual fields were normal (fig. 3).

Dark-adaptation curve. Normal curve with cone and rod segments clearly separated by a "break" at five to six minutes (fig. 5).

Rentinal profiles. Red, blue, and white light thresholds were all within normal limits at each point tested (fig. 5).

Electroretinogram. The light-adapted and dark-adapted responses were normal in amplitude and shape. The presence of a large scotopic response was shown by the marked increase in negative and positive deflections in the dark-adapted state as compared to light adaptation. (See figure 9 for original records, and Table 1 for amplitude measurements.)

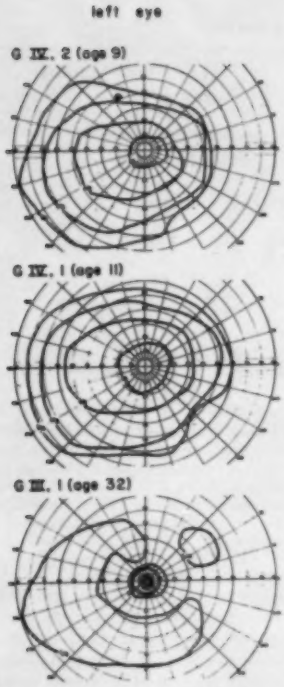

Fig. 3 (Goodman and Gunkel). Peripheral visual fields from the left eye of three members of the G family. The fields were recorded on the illuminated hemisphere of the Goldmann projection perimeter using the 1/4 mm² target at four intensities—full, 0.31, 0.09, and 0.03—which are designated respectively 1/4, 1/3, 1/2, and 1/1. The normal isopter for 1/4 is charted in Figure 4. G III 1 showed changes typical of retinitis pigmentosa: a ring scotoma with breakthrough to the periphery with the brightest target (1/4), and a retention of a central island of vision with dimmer targets. G IV 1 is an unaffected subject with normal fields. G IV 2 is night-blind, but her visual fields at the age of nine years are still essentially normal.

This patient's electroretinograms with red and blue light flashes are also illustrated as an example of normal tracings (fig. 13).

G IV 2

The parents of this nine-year-old schoolgirl stated that they detected poor night vision in this child as early as three months of age. Because of the family history of night-blindness, each of their children was tested in infancy by advancing toys to them in a darkened room. Whereas the two other children responded normally, this subject was said to be less aware of the objects until they were brought close to her central line of vision. The subject herself volunteered no ocular complaints, and it remained uncertain whether she was aware of any deficiency in night vision.

Visual acuity. 20/20, O.U., uncorrected.

Color vision. Normal.

Anterior segments. No abnormalities.

Fundi. There was a thin ring of peripapillary

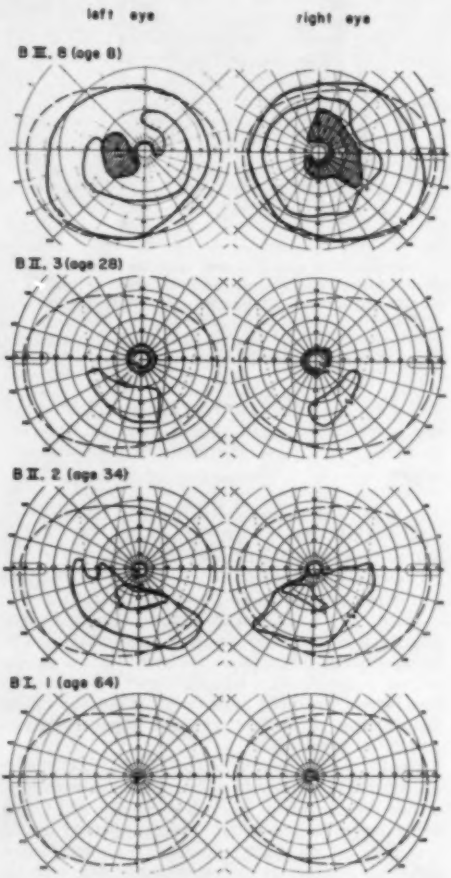

Fig. 4 (Goodman and Gunkel). Peripheral visual fields in four members of the B family with retinitis pigmentosa. The fields were recorded on the illuminated hemisphere of the Goldmann projection perimeter using the 1/4 mm.² target at two intensities—full and 0.31—labelled 1/4 and 1/3 respectively. The normal isopter for 1/4 is represented by an interrupted line. The field changes are typical of retinitis pigmentosa, ranging from incomplete ring scotomas with and without breakthrough to the periphery (III 8), to central islands of vision with retained segments of peripheral field (II 2 and 3), and those without remnants of peripheral field (I 1).

chorioretinal atrophy similar to that seen in her father (G III 1) though narrower in width. Discs and maculas were normal, and there were no abnormal pigment deposits. The vessels were definitely within normal limits, though they were not as full as her sister's (G IV 1).

Visual fields. Central visual fields were full to a 1.0 mm. white object at one meter. Peripheral fields were within normal limits with target 1 at four intensity levels, but the isopters were consistently about five to 10 degrees smaller than those of the normal sister (fig. 3). It was uncertain whether this represented a true difference, or whether it was attributable to more accurate reporting on the part of the older sister.

Dark-adaptation curve. The cone segment of the curve was normal, the "break" was delayed to 12 minutes, and the scotopic increment was reduced so that after 25 minutes of dark adaptation the threshold was $10^{0.6}$ μμl—about 1.5 log units above normal (fig. 5).

Retinal profiles. All thresholds were elevated above normal except for the red thresholds at fixation and two degrees above (fig. 5). The red threshold rose gradually to the periphery from fixation, and a similar course was followed by the white and blue profiles from two degrees to the periphery. Blue thresholds deviated from the normal more than the red thresholds. Retinal profiles were also obtained for the inferior retina from zero to 40 degrees and were similar to those in superior retina except for a greater elevation of thresholds toward the periphery (fig. 7).

Electroretinogram. The light-adapted response

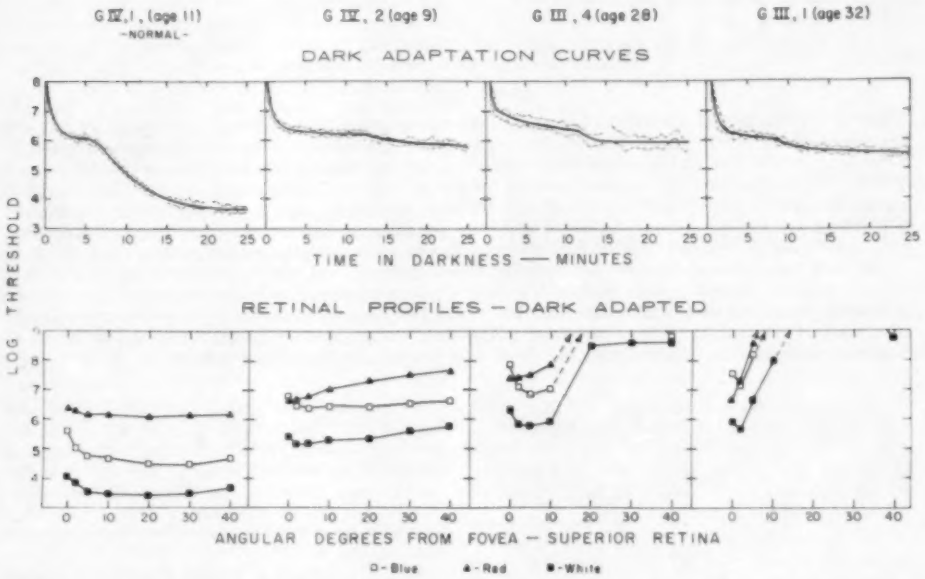

Fig. 5 (Goodman and Gunkel). Dark-adaptation curves and retinal profiles from the right eye of a normal and three affected members of the G family. The range of normal values for both tests is shown in Figure 6.

The dark-adaptation curve was obtained with an 11-degree white light test patch centered 11 degrees below fixation (that is, superior retina). Log threshold refers to brightness in micromicrolamberts (μμl). The upper broken lines connect points representing thresholds of disappearance, and the lower broken lines are for thresholds of disappearance. These thresholds were measured continuously at a rate of about three to five determinations per minute, except where gaps in the broken lines indicate a rest period. Each affected subject showed elevation of the second portion (rod segment) of the dark adaptation curve while the first portion (cone segment) was within normal limits or only slightly elevated (III 4).

Retinal profiles were measured with two-degree white, red, and blue lights from zero to 40 degrees along the vertical meridian of superior retina. Log threshold for the white light refers to μμl; the thresholds for red and blue are arbitrary values. Each affected subject showed a greater elevation of blue thresholds above the norm than red, indicating a predominating rod dysfunction. The central 10 degrees of field showed relative sparing, there was a reversal of the normal fovea-to-periphery relationship, and there was evidence of localized regional elevations of threshold analogous to ring scotomas on visual field testing. See text for discussion.

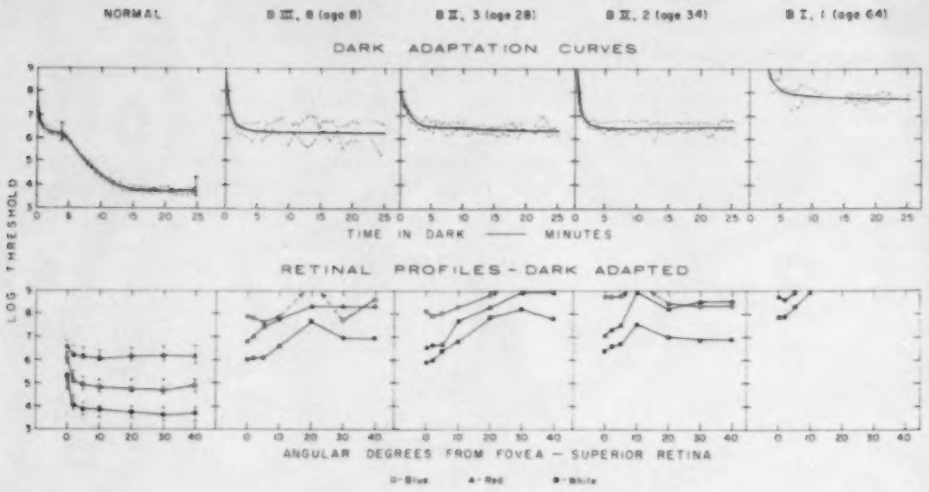

Fig. 6 (Goodman and Gunkel). Dark-adaptation curves and retinal profiles in a normal subject and four members of the B family with retinitis pigmentosa. The range of threshold determinations for 10 normals after four and 25 minutes of dark adaptation, and for five normals on retinal profile testing, are indicated by vertical bars (I) superimposed on the curves obtained in the normal subject. The thresholds for the adaptation studies, and the retinal profiles with white light, are in μμl; thresholds for red and blue retinal profiles are arbitrary values. For details regarding technique and method of plotting data see text and Figure 5 (legend). All affected subjects showed absence of the second portion (rod segment) of the dark adaptation curves; the first portion (cone segment) was within normal limits for the three younger subjects. Retinal profiles revealed a greater elevation of blue thresholds above the norm than red, indicating a predominating rod dysfunction. There was relative sparing of the central 10 degrees of field, a reversal of the normal fovea-to-periphery relationship, and evidence of localized regional elevations of threshold analogous to ring scotomas on visual field testing. See text for discussion.

showed a high normal amplitude, but the dark-adapted response was subnormal. (See Figure 9 for original records, and Table 1 for amplitude measurements.) The scotopic deficit was more apparent in the responses to red and blue light stimuli of weak and moderate intensity (fig. 13).

B Family

The pedigree of the B family is presented in Figure 2. The propositus (B II 2) is a 34-year-old white man who was referred to the National Institutes of Health with a diagnosis of retinitis pigmentosa. He reported that his younger sister (B II 3) and mother (B I 1) suffered from night-blindness, with the latter now complaining of restricted daytime vision as well. He has three children, and did not think that they had any visual difficulty at night. The affected sister thought that daughter B III 9 might have had some difficulty. A brother of the propositus (B II 1) was contacted directly. He denied night-blindness for himself and his children, and quoted his ophthalmologist to the effect that all had normal eyegrounds. The mother of the propositus (B I 1) stated that her mother had night-blindness, but this was said to have improved as she grew older. B I 1 was one of five children. The other siblings were all said to have normal night vision. The daughter of one of her brothers, however, had been heard to remark that she could not see well at night. The father of this subject was contacted and denied night-blindness for himself or his daughter. Attempts to contact the daughter directly to confirm her statement were unsuccessful.

A full examination, including electroretinogram, visual fields, adaptometry, and retinal profiles, was completed for the propositus (B II 2), his mother (B I 1), his sister (B II 3), and the sister's oldest child (B III 8). Five children in the third generation (B III 5, 6, 7, 9, 10) were examined clinically and electroretinographically, but they were too young to participate in visual field, adaptometric, or retinal profile studies. B III 5 and 9 co-operated sufficiently for a determination of final light thresholds.

B I 1

This 63-year-old housewife stated that she had poor night vision as far back as she could remember. During the first 30 years of her life she felt that her daytime vision was normal but, by the age of 48 years, side vision was sufficiently constricted to

prevent daytime driving. Since then daytime and night vision have progressively decreased, and lately she had had difficulty in distinguishing shades of color. A definite diagnosis of retinitis pigmentosa was made at the age of 25 years by an ophthalmologist who examined her fundus as part of a routine visit.

Visual acuity. 20/400, O.D., with a −.75D. sph. ⊂ −1.75D. cyl. ax. 105°; 20/200, O.S., with a −1.0D. sph. ⊂ −1.25D. cyl. ax. 80°.

Anterior segments. There were diffuse posterior complicated cataracts and centrally located anterior polar cataracts in both eyes.

Fundi. Fundus examination was difficult because of the lens changes, O.U. However, both fundi showed pale discs, attenuated vessels, peripapillary atrophy, retinal depigmentation alternating with dense irregular pigment deposits, and finer pigment deposits of the typical bone corpuscle shape. The pigmentation was located in the equatorial zone throughout the entire circumference of the retina.

Visual fields. Central visual fields, O.U., with a 10-mm. white target at one meter did not exceed five degrees. Peripheral fields using the largest (64 $mm.^2$) target on the Goldmann perimeter at full intensity showed a central field from five to 10 degrees in size. The field was less than five degrees with the standard 1/4 target (fig. 4).

Dark-adaptation curve. Since the patient could not appreciate the 11-degree target consistently in its usual position, beginning six degrees from fixation, testing was done with this stimulus centered at fixation. No thresholds could be measured during the first three minutes. There was a lowering of threshold of about one log unit between three and eight minutes, and then a flattening of the curve. The threshold at 25 minutes was $10^{1.8}$ μμl—about 3.5 log units above normal. The lack of a "break," and the marked elevation of blue thresholds relative to the red thresholds in retinal profile testing, suggest that the dark adaptation curve was determined by the cone system (fig. 6).

Retinal profiles. White light thresholds could be measured to 10 degrees, and red light thresholds to five degrees; the patient could not see the blue light stimuli anywhere in the field. The lowest thresholds were present at the macula (fig. 6).

Electroretinogram. The electroretinogram was completely extinguished to stimulus 100, even when the opaque diffuser—which reduced light intensity about 40 percent—was removed. (See Figure 10 for original records.)

B II 2

This 34-year-old government employee was first noted to have poor night vision at about the age of seven years. During the past few years he has become aware of slight but definite impairment of visual acuity, and possibly some restriction of side vision.

Visual acuity. O.D., 20/70, with a −1.5D. sph. ⊂ −0.5D. cyl. ax. 165°; O.S., 20/30, with a −1.5D. sph. ⊂ −0.5D. cyl. ax. 55°.

Color vision. Normal.

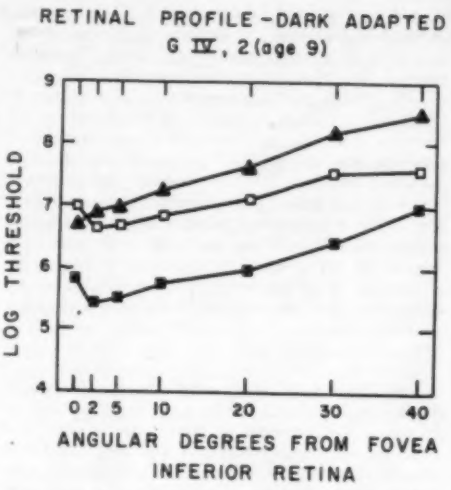

Fig. 7 (Goodman and Gunkel). Retinal profiles for G IV 2 along the vertical meridian of inferior retina in the right eye. Normal retinal profiles are shown in Figure 6. The shape of the profiles was similar to those obtained from this subject in superior retina (fig. 6), but there was a greater elevation of thresholds toward the periphery. □—blue, ▲—red, ■—white.

Anterior segments. There were small round posterior polar lens opacities, O.U.

Fundi. The optic disc showed prominent central cupping. All vessels, temporal as well as nasal, emerged from the nasal side of the disc. (A similar finding was noted in B II 3 and B III 5.) The vessels appeared normal in caliber, or only slightly narrowed. Typical bone corpuscle pigment deposits were found in the equatorial zone of both eyes but limited mainly to the nasal half of retina, O.U. The macular areas were normal.

Visual fields. There was complete absence of the superior field with the 1/4 test object, O.U., on the Goldmann perimeter. An annular scotoma separating an intact central field of vision, and a peripheral segment of field, could be demonstrated with the 1/4 target, O.D., and with the 1/3 target O.S. (fig. 4).

Dark-adaptation curve. The curve was monophasic, reaching a plateau at about five minutes which was maintained at this level for the remainder of the testing period. The final threshold was $10^{0.9}$ μμl—over two log units above normal (fig. 6).

Retinal profiles. All points showed elevated thresholds. Red, blue, and white light profiles revealed a zone of relatively good function up to five degrees from fixation, then an abrupt elevation of thresholds between five and 20 degrees (the region of the ring scotoma), with some improvement of function at 30 and 40 degrees. The elevation of the blue profile above normal was significantly greater

than that of the red at all testing points (fig. 6).

Electroretinogram. A definite light-adapted response was elicited within the lower limits of normal. The dark-adapted response was markedly reduced. With the 12.5 stimulus there was no evidence of a change in shape or amplitude of the response from the light-adapted electroretinogram, showing that no scotopic potential was apparent with this stimulus. A change in shape and amplitude was observed with the 100 stimulus in dark adaptation. This demonstrated some evidence of scotopic activity, although it was markedly depressed. (See Figure 10 for original records, and Table 1 for amplitude measurements.) The dark-adapted response to both blue and red light was a photopic complex (fig. 13).

B II 3

This 28-year-old housewife first became aware of difficulty with night vision at about the age of 10 years. She continued to drive a car at night and felt that her night vision was not very far below normal. She was not aware of constricted side vision during the day. During the previous year she had noted a slight decrease in visual acuity which she attributed to a change in refraction.

Visual acuity. 20/200, O.U., uncorrected. 20/25, O.D., with a −2.25D. sph. ⊃ −1.5D. cyl. ax. 180°; 20/25, O.S., with −2.0D. sph. ⊃ −1.25D. cyl. ax. 180°.

Anterior segments. Posterior polar cataract, O.D., and an incipient posterior polar change, O.S.

Fundi. There was prominent central cupping, O.U., and the temporal and nasal vessels emerged from the nasal border of the cup. The arterioles showed slight narrowing. There was a generalized depigmentation of retina with increased visibility of choroidal vessels. The bone corpuscle pigment deposits were confined to the lower half of the retina, O.S., and to the inferior nasal quadrant, O.D. The macular areas were normal.

Visual fields. The central visual field was full with a five-mm. white object at one meter but restricted to 12 degrees with a three-mm. object. Peripheral fields with a 1/4 target showed a central island of vision 10 degrees in diameter and an inferior temporal segment of field, O.U. The peripheral island of vision was absent with a 1/3 target, and only an eight-degree central field remained. (fig. 4).

Dark-adaptation curve. The curve was monophasic reaching a plateau at about five minutes which was maintained at about this level for the remainder of the stay in darkness. The final threshold was $10^{0.3}$ μμl—about two log units above normal. The curve was similar to that of the brother (B II 2) and son (B III 8) (fig. 6).

Retinal profiles. Except for the red thresholds at zero and two degrees, all other testing points with blue, red, and white light showed elevated thresholds. The curves paralleled each other in showing function best preserved within five degrees from fixation and a fairly sharp rise in threshold to 20 degrees, where the profiles leveled off to 40 degrees. Blue thresholds exhibited greater deviations from the norm than the red thresholds (fig. 6).

Electroretinogram. The light-adapted responses were present, but they were greatly depressed. The dark-adapted response with the 12.5 stimulus showed no change from that in the light-adapted state. With the 100 stimulus the dark-adapted response showed a deeper negative wave, and the appearance of two humps in the depressed positive response. The second hump was a low amplitude scotopic b-wave. (See Figure 10 for original records, and Table 1 for amplitude measurements.)

B III 8

The subject is an eight-year-old schoolboy who denied any visual difficulty. He and his parents stressed the fact that he frequently put the trash out at night and had never stumbled or bumped into objects in the darkened yard.

Visual acuity. Visual acuity was 20/20—2 without correction.

Color vision. Normal.

Anterior segments. No abnormalities.

Fundi. Optic discs and maculas were normal. There was a very mild narrowing of arterioles. The retinal pigment appeared thinned out, and there was a granular appearance to the fundus. One discrete round pigment deposit was present in the retinal periphery of each eye.

Visual fields. Central field examination with a one-mm. white target at one meter showed a field constricted to 10 degrees. The peripheral field examination with a 1/4 target, O.D., was normal, but a scotoma could be elicited between 10 and 30 degrees temporally, O.S. With the 1/3 target the field appeared contracted temporally, O.D., and an incomplete ring scotoma was demonstrated. A contracted field and incomplete ring scotoma with break-through to the periphery was present, O.S., when tested with the 1/3 target (fig. 4).

Dark-adaptation curve. The curve was monophasic reaching a plateau at about five minutes which was maintained for the remainder of the stay in darkness. The final threshold was $10^{0.3}$ μμl—about two log units above normal. The curve was identical with that of the subject's mother (B II 3) and uncle (B II 2) (fig. 6).

Retinal profiles. The only point within normal limits was the macular red threshold, and this was at the uppermost limits of normal. Each curve showed the lowest thresholds between zero and five degrees. The blue and white curves had a localized elevation between five and 30 degrees consistent with the scotoma noted on routine visual field testing. Throughout, the blue thresholds showed a greater average deviation from the norm than the red thresholds (fig. 6).

Electroretinogram. The light-adapted response was normal. The electroretinogram in response to the 12.5 stimulus, dark adapted, showed no change from the light-adapted response, due to an absence of a scotopic negative or positive wave. The dark-adapted response to stimulus 100 was definitely subnormal. The positive wave showed a double-peaked

response, as in B II 2 and B II 3, in which the second wave represented a low amplitude b-wave. These tracings represented a normal photopic electroretinogram and a markedly depressed scotopic electroretinogram. (See Figure 10 for original records, and Table 1 for amplitude measurements.) The response to red and blue light flashes was a photopic diphasic complex (fig. 13).

B III 5

This five-year-old boy was said to have normal day vision and night vision. On further questioning of the parents they recalled that on several occasions the child expressed a fear of going into the street at night but they felt that this probably represented a fear of dark places unrelated to any visual defect.

Visual acuity. 20/20 with "E" game.

Anterior segments. No abnormalities.

Fundi. Normal subalbinotic fundi except for the emergence of all vessels from the nasal side of the disc as in B II 2 and B II 3.

Final light threshold. On two separate occasions the light threshold was measured after 15 minutes in darkness, without prior light adaptation, allowing random fixation and binocular reporting. The 11-degree white light stimulus was used. Ten thresholds were obtained in each testing session over a five minute period which were consistently at about $10^{0.1}$ μμl—and showed no change during the five minutes of testing. This represented an elevation of threshold about 1.5 log units above normal.

Electroretinogram. The light-adapted response in this case was obtained with stimulus 50 and appeared to be within normal limits. The dark-adapted responses were depressed. A small positive stimulus artifact preceded the negative wave in the illustrated tracing, making the negative wave appear deeper than it was on other records from the same patient. (See Figure 10 for original records, and Table 1 for amplitude measurements.)

B III 9

This five-year-old-girl was suspected by her parents to have some difficulty with night vision because she had frequent accidents in the street after twilight.

Visual acuity. 20/20 with "E" game.

Anterior segments. No abnormalities.

Fundi. Normal subalbinotic fundus.

Light thresholds. Light thresholds were measured on two occasions after 15 minutes in darkness, without prior light adaptation, as in subject B III 5. The thresholds were consistently between $10^{0.0}$ to $10^{0.6}$ μμl—about two log units above normal. For one testing session a red threshold was also measured and found to be at $10^{0.5}$ μμl—which is about the upper normal limit for red profiles between zero and 40 degrees in normals. Thus, a decidedly abnormal scotopic threshold and a relatively normal photopic threshold were measured in this patient.

Electroretinogram. The light-adapted response was within normal limits. The dark-adapted responses were subnormal in amplitude. With the 100 stimulus the positive response showed two humps, due to a separation of the early photopic positive response, and the depressed scotopic positive response. (See Figure 10 for original records, and Table 1 for amplitude measurements.)

B III 6

The subject is a three-year-old girl who was said to have normal vision.

Anterior segments. No abnormalities.

Fundi. Normal subalbinotic fundi.

Electroretinogram. The single flash light-adapted response was not tested. However, a 30/second flickering stimulus, which elicits only a photopic response, was delivered in darkness, and the response was within normal limits. The dark-adapted single flash responses were depressed. With stimulus 100, a separation of the photopic and scotopic positive waves was seen similar to B III 9. (See Figure 10 for original records, and Table 1 for measurements.)

B III 7

The subject is a one and one-half-year-old girl. The parents have noted no visual difficulties.

Anterior segment. No abnormalities.

Fundi. Normal subalbinotic fundi.

Electroretinogram. The light-adapted electroretinogram was not tested. The dark-adapted responses were normal in shape and amplitude. (See Figure 10 for original records, and Table 1 for measurements.)

B III 10

The subject is a 16-month-old boy. The parents have noted no visual difficulties.

Anterior segment. No abnormalities.

Fundi. Normal subalbinotic fundi.

Electroretinogram. Normal light-adapted and dark-adapted responses. (See Figure 10 for original records, and Table 1 for measurements.)

Results

1. Dark-adaptation curves (figs. 5 and 6)

Normal dark-adaptation curves are depicted in Figures 5 and 6. They show two segments, separated by a "break" between five and 10 minutes. The first segment of the curve is identified with photopic (cone) function, and the second portion with scotopic (rod) function.[14]

The three affected members of the G family showed both segments of the dark-adaptation curve (Fig. 5). Though the cone segment was within normal limits in two of these subjects (G IV 2 and G III 1), and only slightly altered in a third (G III 4), all showed a rod segment which was elevated

about 1.5 log units at the end of 25 minutes of darkness. In G III 4 and G IV 2, the rod-cone "break" was delayed until 12 to 13 minutes.

All four affected members of the B family demonstrated a monophasic curve. The shape of the curve, lack of a "break," and the marked elevation of blue thresholds noted in the retinal profiles, were evidence that these dark-adaptation curves corresponded to cone segments. These thresholds were within the normal threshold limits of the cone curve in the three youngest subjects, but there was a retardation and elevation of this cone segment in the 64-year-old subject (B I 1). In the latter case the poor thresholds must be attributed in part to the lens opacities, in addition to the retinal pathologic alteration.

The intrafamilial similarity of the adaptation curves is of interest. The difference between the appearance of the curves in the two families is a quantitative rather than a qualitative one, and merely reflects the fact that one group showed sufficient scotopic function within the area tested to result in the appearance of a rod segment, while the second group demonstrated a monophasic curve because rod thresholds were elevated above cone thresholds.

2. Retinal profiles (figs. 5 to 7)

Perimetric light sense studies (retinal profiles) were obtained from seven affected subjects at 0, 2, 5, 10, 20, 30, and 40 degrees from fixation in superior retina, using two degree white, red, and blue stimuli. The log threshold for white light refers to μμl, while the log thresholds for red and blue light are arbitrary values.*

The range of findings in five normal subjects is depicted in Figure 6. With all three stimuli the foveal threshold was elevated above that of extrafoveal points. This is typical of rod threshold contours, since the rods are absent in the fovea, and do not reach their maximum concentration until about 20 degrees from fixation. However, with white and blue light the difference in threshold between the fovea and extrafoveal loci was as much as 1.5 log units, while with red light, to which the rod system is relatively less sensitive, the increase in threshold was usually less than half a log unit. The difference between the effectivity of the blue and red light for the rod and cone systems is graphically shown in their dark-adaptation curves in a normal subject (fig. 8). With red light there was a poorly defined break and a small scotopic increment of half a log unit, while with blue light the cone segment was elevated and shortened, but the scotopic increment was over 2.5 log units.†

The difference between the extrafoveal red and blue thresholds in normals was over one log unit under the conditions of this study. A narrowing of this gap, or an elevation of the blue profile above the red, suggested a relatively greater affection of rod function. For example, if a 1.5 log unit elevation of rod threshold occurred in a retinal area where normal cone function was present, the blue thresholds would show a 1.5 log unit elevation, while the red thresholds would be elevated only 0.5 log unit. This would occur because the cone thresholds with red light are only half a log unit higher than the rod threshold with red light, and with the disappearance of this rod contribu-

* The log thresholds listed for the red and blue stimuli are actually a measure of the brightness of the white light in μμl before passage through the colored filters to elicit a threshold achromatic response. Since no correction was introduced for the change in luminous and spectral transmission produced by the filters, the thresholds represent arbitrary values.

† Wratten red filter No. 29 used in this study transmits sufficient orange light to elicit a scotopic segment in the dark adaptation curve when achromatic thresholds are reported. This varied from about 0.1 to 1.0 log unit in five normal subjects. The scotopic segment with the blue filter varied from about 2.5 to 3.0 log units in these subjects.

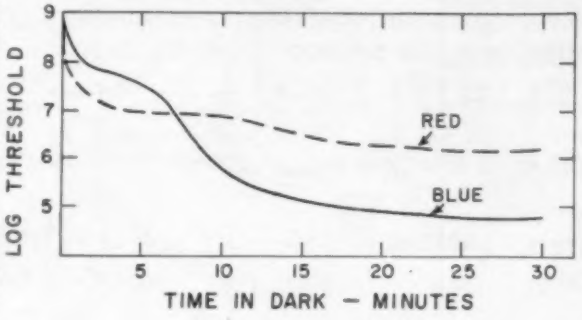

Fig. 8 (Goodman and Gunkel). Dark-adaptation curves in a normal subject with two-degree red and blue lights centered 10 degrees below fixation. The red filter transmits sufficient orange light to demonstrate a "break" and a small scotopic segment. With blue light the cone segment is elevated and shortened; the scotopic portion of the curve is about 2.5 log units. During the first (cone) segment of the curve the red threshold lies below the blue, and during the second (rod) segment, the blue threshold lies below the red.

tion the cone threshold would determine the threshold.

The profiles in all affected subjects were inverted, that is, the fovea and/or parafovea showed the lowest thresholds, with all points beyond five or 10 degrees yielding higher thresholds. In the more advanced cases, peripheral thresholds could not be measured beyond 10 degrees. A minor but consistent familial difference was the fact that the lowest threshold in the B family was usually the fovea itself, while a parafoveal area was always the lowest in the G family. Five subjects (B III 8, B II 2, B II 3, G III 1, and G III 4) showed a localized elevation in one or more of their profiles, bounded on either side by lower thresholds, which were analogous to "ring scotomas." No such localized break in the profile could be demonstrated in G IV 2, who represented the affected subject with the mildest defect (figs. 5 and 7). In the subject with the most advanced pathologic process no thresholds could be measured beyond 10 degrees.

All points tested in each patient showed an elevation of the blue threshold above the norm which was greater than that of the red, demonstrating a relatively greater depression of the rod system. The only normal thresholds recorded were for red in the foveal or parafoveal regions of four subjects (B III 8, B II 3, G IV 2, and G III 1) and the white light macular threshold of G IV 2. In the most seriously affected patient (B I 1) red thresholds could be measured out to 10 degrees, while the blue thresholds were not seen anywhere in the field. It is important to note that the relation between the rod and cone profiles did not vary in different areas, that is, the red and blue curves tended to run parallel. Thus, the areas of localized elevations (ring scotomata) usually showed a symmetric elevation of both the red and blue thresholds, while the parafoveal areas demonstrated a fairly symmetric lowering of both thresholds.

Zeavin and Wald[18] used orange and blue filters for the study of rod and cone retinal profiles in retinitis pigmentosa, and their manner of interpreting the results may also be applied to this study. In comparing the dark-adaptation curves obtained with their filters, they pointed out the fact that the thresholds for orange lay below those for blue during the cone segment of the curve, while the blue thresholds lay below the orange in the rod curve. Therefore, whenever blue thresholds were above orange thresholds in the retinal profiles, this indicated not only a greater depression of the rods than the cones, but the fact that the rod deficit was severe enough so that the dark-adapted thresholds showed the characteristics of cone vision. A similar approach may be adopted in the present study since the same relationship can be shown to exist between the red and blue thresholds during dark adaptation that was found by

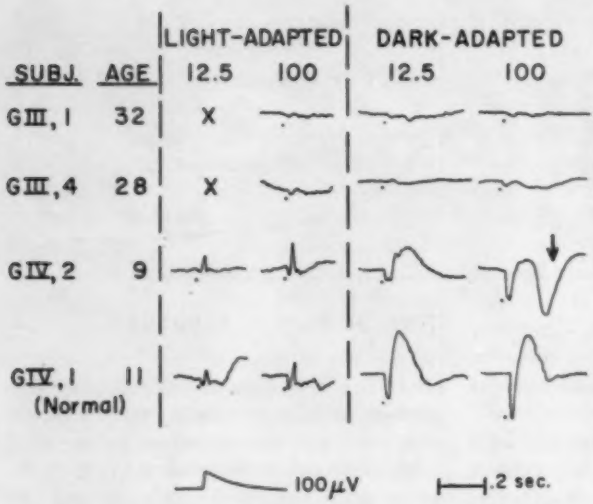

Fig. 9 (Goodman and Gunkel). Electroretinograms in members of the G family with white light stimuli of two intensities (12.5 and 100) in the light- and dark-adapted states. (An X indicates a response which was not tested.) G III 1 and G III 4 show marked depression of all responses. G II 2 has a normal light-adapted tracing but depressed dark-adapted tracings. The scotopic b-wave in G IV 2 is small enough with intensity 12.5 so that the x-wave is not enveloped by it, as in normal subject G IV 1, but appears as an early positive peak. The arrow points to a wink artefact. See text for detailed analysis of these records, and Table 1 for measurements.

Zeavin and Wald with their colored stimuli (fig. 8).* The application to the analysis of results in the two families is particularly interesting, since the B family in almost all points tested revealed red thresholds below the blue, thus indicating that cone vision was actually the determinant of vision in the dark-adapted state. The G family, however, consistently reported the blue thresholds below the red. Here, although the rod deficiency was greater than that of the cones, the characteristics of vision in the dark-adapted state were still scotopic in nature.

3. ELECTRORETINOGRAPHY (figs. 9 to 13)

Recent studies have demonstrated that the human electroretinogram can be differentiated into photopic and scotopic components by appropriate changes in intensity, wavelength, and frequency of stimulation, and the state of adaptation.[17-23] This information was utilized to establish a regimen of clinical electroretinographic testing, outlined in a previous publication,[24] which allowed a separate evaluation of the cone and rod responses. A shortened version of this testing protocol was used in the present study.

Normal electroretinogram tracings, under the conditions of this study, are illustrated in Figures 9 and 10. The light-adapted response to a moderate and strong white light stimulus (intensities 12.5 and 100) was a diphasic response consisting of an initial negative deflection (a-wave) followed by a pointed positive deflection. Both components were present, but decreased in size, with the weaker stimulus. This was essentially a photopic response, since it has been shown that this wave complex is absent in the totally color blind subject,[25-28] present in the con-

* Red and blue dark-adaptation curves were tested with two-degree stimuli in a parafoveal area of five normal subjects, all of whom showed the cone segment of the blue curve elevated from 0.5 to over 1.0 log unit above the cone segment of the red curve (See fig. 8 for curves in a normal subject).

It was expected that a similar relation between the red and blue thresholds would exist in the macular area, where only cones are present. However, retinal profiles in normals consistently showed the blue macular thresholds to be the same as, or lower than, the red. It appeared likely that this finding was due to poor fixation and/or the size of the stimulus patch which allowed stimulation of parafoveal rods and elicited lower thresholds for blue than would be expected from cone receptors. This hypothesis was tested by determining macular blue and red thresholds with one degree targets, instead of two degree, and special attention to good central fixation. Under these circumstances there was 0.5 to 1.0 log unit elevation of blue macular thresholds over the red in normal subjects.

genital night blind[29–31] and Oguchi's disease,[32–33] and that it follows rates of flickering above the fusion frequency of the rods.[23, 26, 29] The positive component had the appearance and temporal characteristics of the photopic x-wave,[21] and will be so designated in this study. The amplitude of this wave, measured from the trough of the a-wave to the first positive peak, served as an index of photopic function. The amplitude of the negative component, the photopic a-wave, was also a measure of the photopic response, but its small size made it a less useful guide than the x-wave. In most cases

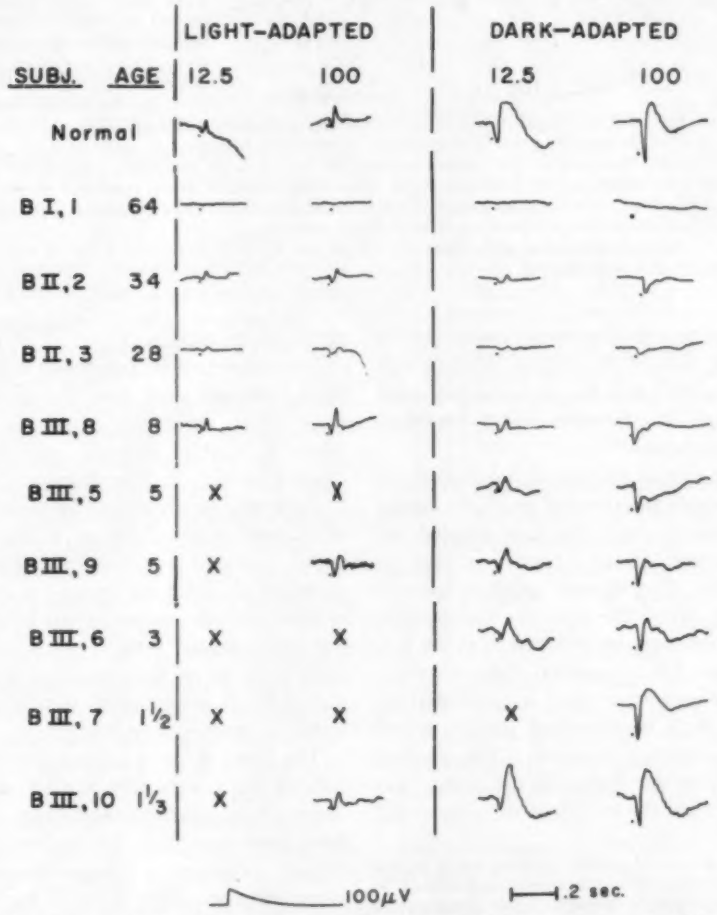

Fig. 10 (Goodman and Gunkel). Electroretinographic records from one normal subject and nine members of the B family with white light stimuli of two intensities (12.5 and 100) in the light- and dark-adapted states. (An X indicates a response which was not tested.) No response is elicited in B I 1 under any conditions. B III 10, 9, and 8 show light-adapted responses within normal limits; B II 2 is a borderline normal. Normal photopic electroretinograms were elicited in B III 6 and B III 5 under different conditions of stimulation, so they could not be included in this chart. Dark-adapted responses were depressed in all subjects three years of age and older even where the light-adapted response was normal, demonstrating the earlier and more severe deterioration of the scotopic system in retinitis pigmentosa. See text for a detailed analysis of these records and Table 1 for measurements.

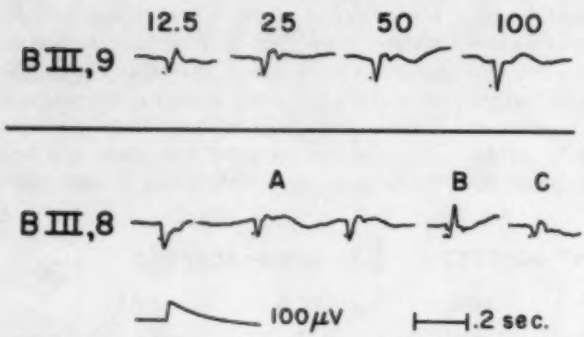

Fig. 11 (Goodman and Gunkel). B III 9. Electroretinographic responses to different stimulus intensities after 20 minutes of dark adaptation. With stronger stimuli the a-wave deepens, and a second hump—the crest of the scotopic b-wave—becomes more prominent until it projects above the peak of the first wave (photopic x-wave). The first wave shows a sharp peak with intensity 12.5 which appears flatter with the higher intensities. In normal subjects with these stimuli the large size of the b-wave prevents identification of the x-wave as a separate peak. (See Figures 9 and 10 for normal responses to intensity 12.5 and 100.)

B III 8. A. The electroretinogram in response to intensity 100 flickering at a rate of 3/sec., in complete darkness. Whereas, the peak of the x-wave is partially hidden in the ascending limb of the scotopic b-wave in the first response, the increased light adaptation resulting from repetitive stimuli depresses the b-wave and allows the x-wave to project above it in the second and third responses. The a-wave undergoes a parallel reduction due to depression of its scotopic component.

(B and C) Electroretinogram with intensity 100 in the light-adapted state prior to (B) and after (C) a 30-minute stay in darkness.

there was a small positive hump in the descending limb of the x-wave. There is evidence to suggest that this is a scotopic wave, even though it is present under conditions of light adaptation.[34]

The amplitude of the dark-adapted response, in both positive and negatives waves, was increased over the light-adapted response because of the intrusion of scotopic components. The scotopic positive wave is commonly called the b-wave; the scotopic negative wave will be referred to as the scotopic a-wave.* A comparison of the two dark-adapted responses revealed a substantial increase of both negative and positive waves with the stronger stimulus. (The positive wave, as in the light-adapted state, was measured from the trough of the a-wave and not from the baseline.)

It is important to remember that *with the strong stimuli used here the dark-adapted response is not purely a scotopic response* since a sizable photopic contribution is "buried" within it. The photopic a-wave and x-wave may be unmasked by means of light adaptation in the form of a brighter surround, or rapid repetitive light stimuli, both of which diminish the scotopic response. An isolated scotopic response can be elicited in the dark-adapted state if weak stimuli are used, since the photopic response has a higher threshold of stimulation, but such stimuli were not employed in this study.

The lower limit of normal for the amplitude of the x-wave with the 100 stimulus is about 75 μv. Since values as high as 200 μv have been recorded, this lower value may actually represent a subnormal response in some subjects. (See Table 1 for measurements of electroretinographic amplitudes.)

All subjects below the age of nine years in whom the photopic response was recorded showed an amplitude within the normal range. The five adults in this study were 28 years of age or older, and only B II 2 showed a photopic response within normal

* The photopic and scotopic positive waves in this study are designated as the x-wave and b-wave as they were originally named. Some investigators prefer the labels b_1 and b_2 which serve to emphasize the earlier appearance of the photopic positive potential. In the case of the a-wave, the symbols a_1 and a_2 have also been suggested for the photopic and scotopic components respectively.[35] This terminology for the a-wave has not been used here because it may imply that the two negative components run a different temporal course, and this has not been proven.[36]

limits. The oldest affected subject (B I 1) gave no response, while the other three adults showed a markedly depressed response. In general, the photopic a- and x-waves ran a parallel course in all patients. These electroretinographic findings demonstrated a progressive deterioration of the photopic response in the later stages of the disease, with retention of normal photopic amplitudes in the early stages.

The lower normal limit for the dark-adapted positive response in this laboratory is about 240 μv for the 12.5 stimulus, and 300 μv for the 100 stimulus. The electroretinograms in B III 10 (16 months), B III 7 (18 months), and G II (11 years) were well above these values. All subjects from three years of age and older, except normal subject G II 1, demonstrated abnormal dark-adapted responses. In both families the response tended to be progressively reduced with increasing age.

It is significant that in both families the abnormal dark-adapted electroretinograms appeared earlier than the abnormal light-adapted electroretinograms. The light-adapted response was not recorded for subjects B III 6 and 7. However, B III 9, B III 8, and G IV 2 all showed depressed dark-adapted tracings in the face of normal light-adapted tracings.

A demonstration of the relative sparing of the photopic response, and early and more severe affection of the scotopic response, is afforded by a comparison of the amplitudes of the light- and dark-adapted responses in individual subjects. Normally, there is at least a fourfold difference between the amplitude of the positive responses to stimulus 12.5 and a twofold or threefold difference with stimulus 100 in the light- and dark-adapted states. In all subjects above three years of age (except normal subject G IV 1) in whom both responses are recorded, the amplitude of these positive responses approached each other. This was most convincingly demonstrated with the 12.5 tracings, since here the inability to elicit measurable

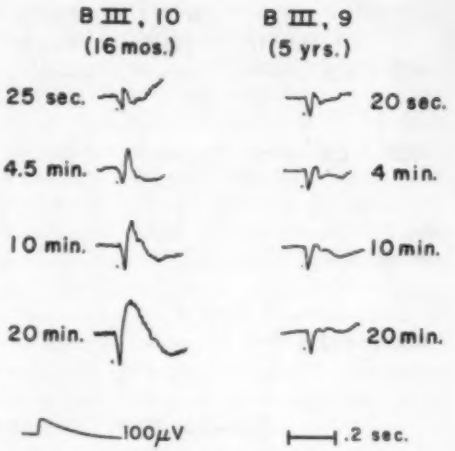

Fig. 12 (Goodman and Gunkel). The electroretinogram in two siblings in response to white light stimulus intensity 100 in the course of dark adaptation after a preliminary light adaptation. In the first half minute, when the response is essentially a photopic one, both electroretinograms are similar. The depression of the scotopic response in B III 9 becomes apparent when compared to B III 10 in the later stages of dark adaptation.

scotopic potentials was shown by an almost identical appearance of the light-adapted and dark-adapted records. The ability of the stronger stimulus (intensity 100) to elicit scotopic a-waves and b-waves resulted in a qualitatively different appearance of the light- and dark-adapted records even when the dark adapted response was much depressed (for example, B III 8). However, in advanced stages, despite the use of the highest intensity, the appearance of the two records was very similar (B II 3, G III 1 and 4),

The presence of two humps in the positive dark-adapted responses of B III 6 and 9 (intensity 100), and G II 2 (intensity 12.5), ascribed to the unmasking of the x-wave, were secondary to the depression of the b-wave. The normal scotopic b-wave, in response to strong stimuli, is of sufficient amplitude and short enough implicit time (that is, time from onset of stimulus to peak of wave) so that it masks the smaller x-wave.

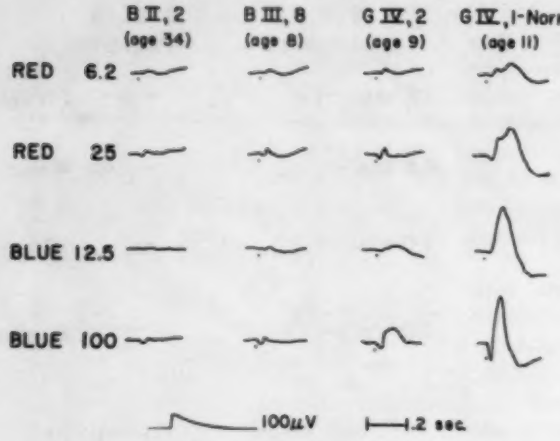

Fig. 13 (Goodman and Gunkel). Electroretinographic tracings from three subjects in different stages of retinitis pigmentosa, and a normal subject, to red and blue stimuli in the dark-adapted state. With red light, the normal subject shows a double-peaked positive response (x-wave and b-wave); the b-wave is lacking in the three affected subjects. With blue light B II 2 and B III 8 still show only photopic responses; G IV 2 is the only affected subject to show a scotopic response, and this is greatly depressed compared to the normal. (Red filter used with intensity 6.2 and 12.5; blue filter used with intensity 12.5 and 100.)

With a reduced b-wave, the x-wave appears as an early positive peak riding on the ascending limb of the b-wave. One can obtain similar curves from normal subjects in the early minutes of dark adaptation, when the b-wave is still sufficiently depressed by the preceding light adaptation.

The type of double-peaked dark-adapted positive response to stimulus 100 found in BIII 8 and B II 2, and suggested in B III 5 and B II 3, differed from that discussed in the preceding paragraph in three ways: the first wave was smaller than the light-adapted positive response, its peak was below that of the second wave which followed it, and the second wave had a greatly prolonged implicit time. What accounted for these differences? The small amplitude of the first wave (x-wave) may be attributed partly to the fact that this photopic positive response decreases in amplitude during dark adaptation. This has been noted in previous studies[21, 27] and could be demonstrated indirectly in B III 8 by comparing the amplitude of the light-adapted response to stimulus 100 to the response to this flash elicited 10 seconds after a 30-minute period of stay in darkness (fig. 11-B and C). The response was much smaller and rounder, the sharp peak present in the ordinary pre-darkness response was barely suggested in this post-darkness response. With the subject remaining in the light-adapted state for several more minutes, and constant stimulation with one per second flashes of stimulus 100, the response became sharper, and increased in amplitude. After five minutes of such stimulation it was almost the same size as the pre-darkness potential. The only difference detectable was in the second small hump, which had been barely visible in the predarkness photopic wave, but was slightly more prominent in the postdarkness response. It seemed that this wave accounted for much of the rounded appearance of the first post-darkness flash, before the x-wave increased in amplitude (fig. 11C).

The decrease in size of the x-wave during dark adaptation is not demonstrable in the normal response because it is enveloped by the b-wave. This phenomenon, however, can be demonstrated in subjects with night-blinding diseases where the b-wave cannot be elicited.[28] When the b-wave was only moderately depressed (for example, B III 6 and 9, intensity 100), the dark-adapted x-wave continued to appear larger than its light-adapted counterpart because it rode on the ascending branch of the b-wave. However, with a decrease in amplitude of

the b-wave, and a lengthening of implicit time, the more rapid x-wave reached its peak before the onset of the b-wave, or at the beginning of the ascending phase, so that a large summation of potential did not occur. In such cases it emerged as the smaller of the two waves, and it also showed a reduction in amplitude relative to its light-adapted appearance.

The presence of a prolonged implicit time for the b-wave may be related to its small amplitude. In normal subjects there is an inverse relationship between implicit time and b-wave amplitude, so that a decrease in amplitude of the b-wave secondary to a decreased stimulus intensity is associated with a lengthened implicit time.[38] A similar relationship appears to exist in retinitis pigmentosa, though here the decrease in amplitude is secondary to the retinal pathologic process and not to a reduction in stimulus intensity.

To show the basic relationship between the two types of double-peaked dark-adapted responses, the dark-adapted response of B III 8—in which the first peak (x-wave) was smaller than the second peak (b-wave) on standard testing—was exposed to repetitive light stimuli (fig. 11). With the increase in light adaptation that ensued, the responses showed a decrease in amplitude of the scotopic b-wave such that the x-wave now projected above the level of the b-wave. (The x-wave also projected above the baseline with this change in adaptation because of the reduction of the scotopic a-wave.) The response now appeared similar to that obtained in B III 9 (fig. 10). Conversely, the transition to the second type of dark-adapted response (that is, second peak smaller than first peak) was demonstrated in B III 9 by obtaining tracings in the later phases of dark adaptation. Whereas the standard response to stimulus 100 showed the x-wave projecting above the b-wave (fig. 10), the increased amplitude of the b-wave with a longer stay in darkness produced a response in which the b-wave appeared higher than the x-wave (fig. 11-100 and fig. 12).

The growth in amplitude of the dark-adapted electroretinogram after a pre-exposure to light was charted for B III 10, who showed a normal response, and B III 9 with a depressed response (fig. 12). Once again the intactness of the photopic response was manifest. The first response after 20 seconds in darkness, which was still primarily photopic, was similar in the two subjects—even slightly larger in B III 9. The depression of the scotopic b-wave in B III 9 relative to B III 10, already apparent at four minutes in darkness, became more apparent with increasing dark adaptation.

With stimuli of long wavelength the b-wave is reduced in amplitude and its implicit time is sufficiently prolonged so that it may be separated from the preceding photopic x-wave in the dark-adapted response.

Figure 13 shows the electroretinogram in a normal subject (G IV 1) and three affected subjects (B II 2, B II 8, and G IV 2) who represented three different stages of retinitis pigmentosa. The response to red light in the normal showed the characteristic double peak; in the three affected subjects the second peak (b-wave) was absent. With blue light stimuli the normal subject demonstrated a single peaked response: whatever small photopic potential was present was incorporated in the large b-wave. The responses of B II 2 and B III 8 to blue stimuli were small potentials, monophasic with weaker stimuli and diphasic with stronger flashes, which in time course suggested small photopic responses. A small scotopic response, however, was elicited in G IV 2. The b-wave was so depressed in this subject that with blue stimulus 12.5 the photopic wave could still be distinguished as a small ripple on the ascending limb of the b-wave.

The electroretinographic responses to the standard white light stimuli, red and blue flashes, and the examination of the response

in the course of dark-adaptation in two subjects, all demonstrated the earlier and greater deterioration of the scotopic response, as compared to the photopic response, in retinitis pigmentosa.

Discussion

1. General considerations

This investigation developed as part of an interest in the evaluation of rod and cone function in retinal heredodegenerative diseases and the application of this knowledge to clinical and genetic problems. Studies of this type require a battery of tests to survey photopic-scotopic relationships in different areas of visual function.* Normal as well as affected kin must be examined in order to detect subclinical carrier states and early stages of progressive degenerations. This study represented an attempt, on a limited scale, to utilize such an approach in the investigation of two families with dominant retinitis pigmentosa.

Electroretinography and psychophysical testing in the patients affected with retinitis pigmentosa demonstrated that a rod dysfunction preceded the cone dysfunction. As Zeavin and Wald[18] have pointed out, any proposed explanation for the basic defect in this condition must account for the greater vulnerability of the rod system. One possibility discussed in detail by these investigators is that of a local failure in the metabolism of vitamin A, such as the inability to produce the neo-b isomer. This suggestion was based on three facts: rods and cones are primarily affected in both the vitamin deficiency and retinitis pigmentosa; cone opsin unites more rapidly with neo-b retinene than rod opsin, so that a deficiency could result in an early rod dysfunction; the neo-b isomer has thus far been found only in eye tissue, which might explain why a failure in producing this isomer can lead to visual failure without other systemic effects. Noell[39] claims that experimental iodoacetic acid poisoning the mammals produces a retinal degeneration affecting the rod receptors first with histologic findings like that of human retinitis pigmentosa. He attributes these lesions to an interference with retinal glycolytic mechanisms.

The similarity between certain inherited retinal degenerations in mice, rats, and dogs, and human retinitis pigmentosa, is well known.[40] This is best demonstrated in the dog[41,42] which has a mixed rod and cone retina, rather than in the rat or mouse which have essentially rod retinas. A rod degeneration ensues and the dogs become night blind, while still retaining cone receptors and normal day vision. With deterioration of the cones the animals go on to total blindness and a histologic picture similar to human retinitis pigmentosa. The electroretinographic findings in the present study offer new evidence of the functional parallels existing between the canine and human degenerations. In the dog it has been shown that an electroretinographic response may be recorded at an early stage of the disease which soon disappears.[43] Similar conclusions may be deduced from this study of human retinitis pigmentosa, since it was demonstrated that the younger affected subjects had well-developed responses, while affected adults showed greatly diminished to absent responses. No attempt was made in the canine electroretinographic studies to specifically elicit the photopic response. In view of the observations in affected humans, the separate evaluation of the photopic and scotopic electroretinographic response in the dog would be worthwhile.

The electroretinographic findings in the dog suggest that the rod receptors are functioning before they begin to degenerate.[43] Since the rod dysfunction in the affected humans in this study began before subjective testing was possible, the proof of a functional rod system lies in the electroret-

* An example of a comprehensive testing program may be found in the work of François, Verriest, and DeRouck.[8]

inogram, as demonstrated by the well-defined, though subnormal, scotopic responses of B III 6 and 9 (fig. 10). Indeed, as will be noted later, the pattern of progressive electroretinographic loss, and the fair size of the responses just cited as late as age three, suggest that the scotopic electroretinogram may have been normal earlier in life. This impression is further strengthened by the course of the cone affection. The normal day time vision and intact cone receptors found at one stage in the dog, and the normal photopic electroretinogram and good visual fields in the early course of human retinitis pigmentosa, show that these cone receptors are capable of photoreception and electric activity before their ultimate degeneration. There is no reason to believe that the cone deterioration differs from that of the rods except in its later onset, since the areas of retina affected and the histologic features of the rod and cone degenerations are analogous.

The finding of functional integrity of the receptors before their demise fits in well with the prevailing idea of retinitis pigmentosa as an abiotrophy. In the original formulation of the term by Gowers,[44] and its application to retinitis pigmentosa by Treacher Collins,[45] abiotrophy implied a degeneration of tissue after it had undergone normal maturation.

The concept of abiotrophy has changed, and it is now thought that the tissue in its early stages may appear normal because our methods of examination are relatively insensitive, but the basic defect is present at birth and will some day be recognized in its preclinical state. A step in this direction has been made in studies of mice,[47] rats,[48] and dogs[42] with retinal degenerations similar to human retinitis pigmentosa, which demonstrated that the receptors actually did not reach the final stages of differentiation before degeneration began, though their development was complete enough for normal function. On the basis of the electoretinographic studies in canine retinal degenerations previously cited, it would appear that this defect in differentiation is not sufficient to prevent an electroretinographic response.

2. Dark-adaptation curves

Mandelbaum[14] and Jayle[20] have reported quantitative dark-adaptation studies in retinitis pigmentosa. Their findings concur in showing that the characteristic change in this condition is a contraction of the "photochromatic interval," that is, a decrease in the differential threshold between rods and cones as measured between the rod-cone "break" and the final threshold. Frequently the elevation of the rod threshold is sufficient to abolish this interval completely, and the subject remains with a monophasic curve representing cone thresholds exclusively.

The findings in this study fall into the general pattern described above (figs. 5 and 6). Three subjects, all of the G family, showed a photochromatic interval which was markedly reduced; four subjects, all of the B family, demonstrated a monophasic curve. However, the cone segments of the curves were within normal threshold limits in five of the seven patients, whereas normal cone thresholds were reported by Mandelbaum in only one of 17 cases, and by Jayle in four of 21 patients. This may be related to the fact that dominant forms of retinitis pigmentosa are often the mildest in their manifestations.[9]

None of Mandelbaum's patients exhibited a delay in appearance of the rod-cone "break" whereas G IV 2 and G III 4 both showed a delay to about 12 minutes, and three of Jayle's patients demonstrated similar lags in the time of appearance of the "break." Since a rod and cone disturbance characterizes both vitamin-A deficiency and retinitis pigmentosa, it is noteworthy that Patek and Haig[21] observed a similar retardation of the "break" in a study on patients with cirrhosis of the liver which could be reversed by administration of vitamin A.

3. Retinal profiles

The retinal profiles in retinitis pigmentosa illustrated by Sloan,[52] Mandelbaum,[14] Zeavin and Wald,[18] and this investigation (figs. 5 and 6), demonstrated a relative sparing of foveal and parafoveal areas, a tendency to abrupt elevations of threshold in areas marked by ring scotomata on visual field examination, and the inversion of the normal fovea-to-periphery relationship. With the use of red and blue stimuli to separate rod and cone profiles, it has been shown in this study and by others[14, 18] that the pattern of regional involvement just described is true of the cones as well as the rods, though the rod defect is always more severe. Zeavin and Wald noted the similarity between retinal profiles within the same family, and likened them to a "family signature." This was also evident here where the B family showed a greater elevation of blue thresholds compared to red than members of the G family, and all three members of the G family had a small parafoveal region in which thresholds were lower than those at fixation, whereas this was not found in the four members of the B family.

Sloan[52] has emphasized the diagnostic value of retinal profiles (perimetric light sense testing). First, a standard dark-adaptation curve may be normal because of the relative sparing of paracentral retina on which the test patch is usually projected, while scotopic deficiencies can already be discovered in light sense studies which extend more peripherally. A case of this type was reported by Mandelbaum.[14] Secondly, a patient with retinitis pigmentosa may have grossly abnormal retinal profiles while the standard visual field examination is normal or only slightly altered. Sloan cited several cases; subject G IV 2 is another.

The logical explanation for this finding is that cone vision is relatively unaffected when marked scotopic defects are already present. However, though central and peripheral visual fields in subject G IV 2 were within normal limits, retinal profiles with red light showed a gradual elevation of thresholds toward the periphery which was more than could be accounted for by elimination of the small scotopic component obtained with this filter. It must be assumed that the cone deficiency was slight enough so that it was not manifest by significant field changes on routine perimetry, but it could be demonstrated by the more sensitive method of threshold determinations.

The practical usefulness of threshold studies in the diagnosis of night-blinding diseases in children was evident in the two five-year-olds, B III 5 and 9, who were too young for charting visual fields or full retinal profiles. Final thresholds for red, blue, and white light were easily obtained and demonstrated an elevation of scotopic thresholds exceeding two log units.

4. Electroretinography

Microelectrode[53-56] and degeneration[39] studies have demonstrated the importance of the outer retinal layers (that is, rod and cone receptors) in the generation of the normal electroretinogram. The early derangement of the electroretinogram in retinitis pigmentosa is consistent with these findings, since histologic examinations in affected humans, and animals with similar retinal degenerations, show a degeneration and disappearance of the rods and cones as the primary pathology, with the ganglion cell and bipolar layers remaining intact until the most advanced stages.[57, 58] Pigment deposits and narrowing of blood vessels are secondary phenomena which develop after rod and cone deterioration has begun, but may sometimes be completely absent (so-called "retinitis pigmentosa sine pigmento").

The absence of the electroretinogram in a disease where bipolars and ganglion cells are uninvolved does not allow any conclusion to be drawn about their role in the propagation of electroretinographic waves, since the first neuron in the propagation of the visual impulses is already destroyed. There is evidence from other sources to indicate

that the ganglion cell layer contributes nothing to the human electroretinogram as recorded from corneal leads;[59] it is the status of the bipolars which remains unsettled.

On clinical grounds alone it might be expected that the bipolar cells play a role in the generation or maintenance of a normal response, since the electroretinogram is markedly altered in central retinal artery obstruction.[1,60,61] Here, in contrast to retinitis pigmentosa, pathologic changes are found in the bipolar and ganglion cell layers, but spare the receptors. However, one cannot rule out the possibility that the electroretinographic changes in the arterial occlusion reflect some affection of the circulation in the outer retinal layers which is not evident in routine histologic studies.

The human electroretinogram, under current methods of investigation, represents a crude mass response of retinal elements; fairly extensive lesions must be present in order to cause a reduction in the photopic and scotopic response. This may serve to explain the finding of a normal photopic response in a subject such as B III 8 (fig. 10), when subjective tests revealed evidence of cone deterioration. Conversely, the presence of a central island of vision and some degree of residual rod and cone function in peripheral areas, as one finds in advanced retinitis pigmentosa, may not be adequate to elicit a measurable response, just as localized lesions in these areas in an otherwise intact retina are often insufficient to detract from a normal response.

Riggs[62] has offered an alternate explanation for cases of retinitis pigmentosa with some remaining visual function and absent electroretinograms: widespread perforations of the retina resulting in a short-circuiting of the response. However, the fact that the electroretinogram may become extinguished to strong stimuli in chronic vitamin A deficiency,[63] and then show well-defined rod and cone responses after treatment with vitamin A, suggests that an explanation based solely on the presence of a rod and cone dysfunction, without reference to structural abnormalities and short-circuits, may also satisfactorily explain the "absent electroretinogram" in retinitis pigmentosa.[49] Nonetheless, the possibility that some type of retinal short-circuiting is a factor in the electroretinographic depression in retinitis pigmentosa and other retinopathies cannot be excluded.

Recent reviews of electroretinography in clinical diagnosis have concluded that the electroretinogram is absent in retinitis pigmentosa, and may even be extinguished from birth.[9-11] A few cases have been reported in which a small response was obtained,[20,62] or an isolated a-wave;[9,11] these were noted as rare exceptions to the rule. Several patients required a selective amplification of the electroretinogram to demonstrate a response since none was present in routine recording.[63] There has been only one case in which a substantial, though still subnormal, electroretinogram was found.[64] Since the present work differed from the literature in reporting negative and positive responses in eight of nine affected subjects, it became necessary to critically review these cases in order to be certain that the diagnosis of primary retinitis pigmentosa was merited.

The adult members of the B and G families who were studied all showed a fundus picture of retinitis pigmentosa, characteristic visual fields, dark adaptation curves, and retinal profiles. One eight-year-old (B III 8) showed retinal vascular attenuation and typical functional deficits. These subjects represented six undisputed cases of retinitis pigmentosa.

The presence of normal visual fields, and a history of night-blindness since birth in G IV 2, a nine-year-old girl, raised the question whether this might represent a case of congenital night-blindness. Such a diagnosis presupposes a normal fundus, normal photopic function, and a severe scotopic defect.[11]

The reasons for considering this a case of retinitis pigmentosa rather than congenital

night-blindness may be listed as follows:

1. The fundus showed a ring of peripapillary atrophy. This is frequently seen in progressive tapetoretinal degenerations,[68] and, most important, it was present in more advanced form in the patient's father (G III 1), a man with classical retinitis pigmentosa.

2. There was evidence of photopic loss in the form of elevated red thresholds in the retinal profiles tested inferiorly as well as superiorly.

3. Dark-adaptation curves, retinal profiles, and the electroretinogram all revealed a fair amount of residual scotopic function. The findings in the subjective tests in particular were not in keeping with the severity of the scotopic defect found in most other cases of congenital night-blindness in this laboratory, and those reported in the literature. They were consistent with findings that could be expected in an early stage of retinitis pigmentosa.

4. The dark-adaptation studies and retinal profiles showed patterns strikingly similar to those obtained in the affected father and aunt. This fact was not only significant in itself, but it also suggested that the dip in retinal profiles parafoveally and the gradual rise peripherally in this patient—viewed in the light of the profiles in the other two subjects—probably represented an early stage in the development of a ring scotoma.

5. A history of night-blindness since earliest childhood is often elicited in retinitis pigmentosa, and need not be considered a point in favor of congenital night-blindness.

Three younger members of the B family (B III 5, 6, and 9) had normal fundi, and a depressed dark-adapted electroretinogram; in two cases (III 6 and 9) the existence of a scotopic deficit was confirmed by means of light threshold determinations. Once again the findings raised the possibility of congenital night-blindness. Since visual field and retinal profiles could not be obtained from these patients, the electroretinogram was studied for clues to the diagnosis. The presence of a poor response in the two older children, as compared to the younger, coupled with similar evidence of an increasing electroretinographic deficit in adult affected members, suggested that these children had a progressive disease and not a stationary anomaly. If the latter were the case, then the youngsters should probably have had similar electroretinograms, particularly B III 5 and 6, who were from the same sibship. Instead, the difference in size and shape of response between B III 5 and the other two subjects was particularly striking.

The most potent argument for the diagnosis of retinitis pigmentosa in the subjects with functional defects and without fundus change is the rarity of the co-existence of congenital night-blindness and retinitis pigmentosa. Even the few reported cases are suspect, and in most cases are believed to represent children in the early stages of retinitis pigmentosa, or cases of very mild retinitis pigmentosa.[11] Furthermore, it is a well known fact the retinitis pigmentosa of dominant transmission, like so many other dominant hereditary affections, is a milder and more slowly progressive disease than recessive forms.[9] It is precisely in such families that scotopic defects may precede fundus change and photopic defects, and erroneously suggest the diagnosis of congenital night-blindness.[11] Since both young and old showed a characteristic intrafamilial pattern of progressive visual loss, with the classical fundus findings and functional deficits present in the more advanced cases, the diagnosis in the children with abnormal electroretinograms and normal fundi was overwhelmingly in favor of retinitis pigmentosa in its early stages.

What accounts for the consistent lack of electroretinographic response in retinitis pigmentosa reported in the literature, and the demonstration of sizable responses in most of the patients in this study? A tentative explanation is offered in terms of the difference in light stimuli and patient material in this and early investigations.

The light stimulus used here was the in-

tense flash from a xenon discharge lamp which could elicit light-adapted (that is, photopic) responses in normal subjects with positive waves frequently exceeding 150 μv in amplitude. Most of the earlier studies of the electroretinogram in retinitis pigmentosa dealt with weaker stimuli capable of eliciting only a rod response; the photopic response, whose threshold is about two to three log units above the scotopic b-wave, was insignificant or absent.[59, 66, 67] Since retinitis pigmentosa is marked by a severe rod dysfunction which may elevate the thresholds of the rod electroretinogram above that of the cone electroretinogram even in childhood (for example fig. 10, B III 8, intensity 12.5), the absence of an electroretinographic response to weak stimuli becomes understandable. It is significant that the largest reported response in retinitis pigmentosa was elicited with a light stimulus similar to the one used in this study,[46] while the responses reported by Armington[30] and Riggs[62] were also obtained with strong stimuli capable of eliciting photopic potentials in normal subjects.

The deterioration of vision in retinitis pigmentosa is more pronounced in older patients, those with a recessive type of inheritance, cases with associated neurologic or aural pathology, and patients who already manifest fundus changes. Many of the patients previously studied came from these groups, where the retinal degeneration was more advanced and therefore more apparent. Two studies which give detailed information regarding these points, and which may be analyzed from this standpoint, are those of Karpe[2] and François.[8]

Only seven of Karpe's 29 cases of retinitis pigmentosa with extinguished electroretinograms were below the age of 20 years, and, of these, three were deaf and dumb. Four children from families with dominant retinitis pigmentosa, all below the age of 14, also demonstrated extinguished electroretinograms. Three of these patients already showed evidence of retinal degeneration, though without bone corpuscle pigmentation, at the time of the electroretinographic examination. A seven-month-old child, the youngest recorded in the literature with an extinguished electroretinogram, showed a suggestion of retinal depigmentation even at this early age, and a progression of these changes was clearly evident at the age of three years. François's study comprised 19 subjects, of which six were below 20 years of age, none had a history suggesting dominant transmission, and all had significant fundus alterations and visual field changes. One subject, a 55-year-old man, showed a minimal positive response in one eye.

With the increasing use of high intensity stroboscopic light flashes in this laboratory and others,[69-71b] a number of unreported cases of established retinitis pigmentosa in adults have shown measurable electroretinographic responses. However, even these stimuli were ineffective in obtaining responses in the majority of such cases using current methods of amplification and recording. The high incidence of electroretinographic responses in this study is probably due to the combination of an intense light source, testing in childhood, and the slower course of the degeneration in the two families examined. It must be stressed, however, that in the children below five years of age moderate stimuli sufficed to elicit fair electroretinograms. Thus, even with the use of weak stimuli, good responses might conceivably be obtained in the earliest stages; this is also suggested by the findings of Franceschetti discussed in the next paragraph.

The results of this study were anticipated by Franceschetti and Dieterle[9] in their report of the subnormal electroretinograms in two children, aged eight and 10 years, from a family with a five-generation history of dominant retinitis pigmentosa. The visual fields were slightly constricted, the dark adaptation curves revealed almost complete absence of the rod segment, funduscopy was negative, and electroretinograms one-half and one-third normal size were obtained.

(These responses were elicited with a stroboscopic light source of low intensity, as manifest by a maximum positive response in normals of 180 μv in the undilated eye and 260 μv in the dilated eye.)

Despite the fact that the authors cited the "benign" course of dominant retinitis pigmentosa, they leaned toward a diagnosis of congenital night-blindness, because there had been no previous reports in the literature at this time of electroretinograms in retinitis pigmentosa with only moderate reduction of the response, and 50 cases of primary retinitis pigmentosa studied in their laboratory had shown absent responses.

The findings in the present study would suggest that these two patients represented early stages of retinitis pigmentosa, rather than the very rare finding of congenital night-blindness and retinitis pigmentosa in one family. François[71] has also found a subnormal electroretinogram in one of three affected children in a family with dominant retinitis pigmentosa.

The electroretinogram has been cited as a means of differentiating congenital stationary night-blindness from primary retinitis pigmentosa.[31] This is based on the fact that reported cases of congenital night-blindness have all shown some response to stimuli capable of eliciting cone potentials, whereas previous studies of retinitis pigmentosa showed absent or negligible responses.

Certainly, the finding of an extinguished electroretinogram with adequate stimuli, or the presence of an isolated photopic complex which is markedly depressed, would seem to favor the diagnosis of a progressive retinal degeneration such as retinitis pigmentosa. However, the fact that well-formed responses can be present in retinitis pigmentosa, particularly in early cases when the lack of fundus changes may suggest congenital night-blindness, weakens the value of the electroretinogram as a means of differentiating between the two conditions. Furthermore, an analysis of the electroretinograms reported in congenital night-blindness, as well as new cases studied in this laboratory, shows that the response may vary from one which is primarily photopic, to others in which a scotopic response is also present.* A similar gamut of electroretinographic patterns has been found in the different stages of retinitis pigmentosa in this study.

The difficulty in establishing the correct diagnosis in night-blinding diseases with atypical features, despite careful subjective and electroretinographic testing, is reflected in subject M. G., a patient previously reported as a case of congenital night-blindness.[23, 24, 28] The original diagnosis was based on the fact that this 30-year-old woman, with a complaint of nonprogressive night-blindness from early childhood, had normal fundi, full visual fields with a three-mm. white object at one third of a meter, and absence of the scotopic segment of the dark adaptation curve.

Electroretinography was performed before the results of this study were known, and the presence of an electroretinographic response appeared to be confirmatory evidence that this was indeed a case of congenital night-blindness. Because of the findings in this investigation, the patient was recalled for further testing.

Quantitative peripheral visual fields were done which revealed a ring scotoma; this was confirmed by demonstrating a localized elevation of thresholds on retinal profile testing. Though these findings would suggest that this case is one of retinitis pigmentosa sine pigmento with a particularly mild course, even this diagnosis cannot be accepted with certainty because of the unique electroretinographic findings.

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* Cases reported by Bornschein and Schubert,[29] Armington,[30] and François et al.[31] appeared to be purely photopic in nature with the stimuli used in these studies. Scotopic potentials were evident in two members of a family with dominant congenital night-blindness reported in several publications.[60, 72, 73] Two new members of this family were recently studied in this laboratory and exhibited significant scotopic responses to stimuli 12.5 and 100.

The electroretinographic response, investigated quantitatively in its behavior to changing stimulus intensity and adaptation,[28] was composed of a normal a-wave (that is, scotopic as well as photopic comments), and a photopic x-wave. The absence of the scotopic b-wave, with retention of a completely normal scotopic a-wave, has never been demonstrated in a confirmed case of retinitis pigmentosa. Patients like M. G., with atypical retinal anomalies or degenerations, must be followed with functional testing for many years before an accurate evaluation can be made of their condition, and of the significance of the electroretinographic response.

An advantage of the electroretinogram in the study of retinal degenerations is the fact that it can be employed in young children, mentally retarded subjects, and other patients who cannot co-operate in subjective testing. This is of importance in several respects. It may be useful in genetic counselling, particularly when advising married adults, without a family history of consanguinity, who represent isolated cases of retinitis pigmentosa. In such a situation it is uncertain whether the condition is a dominant mutation, with a 50:50 chance of affected offspring, or whether it represents a homozygous recessive state where the chance of bearing affected children is negligible. If an abnormal electroretinogram is detected in the first born child within the first few years of life, the eugenic implications of this finding can be faced *before* new children have arrived. Ideally, serial electroretinogram testing should be done in these children to detect the first sign of abnormal response. Early diagnosis is important in case treatment becomes available; the electroretinogram might also be useful as an indicator of therapeutic response.

In the clinical examination of the children in this study, special attention was directed to detecting any irregularity in appearance of the pigment epithelium, since slight granularity of stippling of this layer is said to be the earliest fundus sign of retinitis pigmentosa.[74] However, such changes are indistinguishable from the normal appearance of a subalbinotic fundus, and their presence in the fundi of the fair-haired children in this study could not be construed as definite evidence of an incipient retinal degeneration. Thus, despite a high index of suspicion for early changes, normal fundi were found in subjects where appropriate functional testing could elicit evidence of the disease.

Summary

Visual function was studied in 13 members of two families with dominant retinitis pigmentosa who ranged in age from 16 months to 64 years. Electroretinograms were obtained from all subjects, and adaptometry, perimetric light sense testing (retinal profiles), and visual field examinations were completed in those above five years of age. Techniques were utilized in the electroretinographic and subjective tests which made possible a separate evaluation of photopic and scotopic function.

A scotopic deficit, with an intact photopic response, was elicited in younger affected members. There was a progressive deterioration of photopic and scotopic function with advancing age. A significant clinical finding was the presence of depressed scotopic electroretinograms in four children below 10 years of age who showed no pathologic alteration of the fundus. The electroretinogram was extinguished only in the 64-year-old subject, in contrast to previous reports of absent responses in the majority of patients with retinitis pigmentosa.

The physiologic, clinical, and genetic implications of this study are discussed. Stress is laid on the correlation of the electroretinographic findings with modern concepts of the photopic-scotopic nature of the positive and negative waves.

Acknowledgment

We are indebted to Dr. Ludwig von Sallmann for serving as clinical consultant in this study.

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Discussion

Fuortes: There are many experts on electroretinography and maybe some of them will make some comments. Dr. Jacobson.

Jacobson: The findings presented by Dr. Goodman are very significant and I think they point out a number of factors which are of great interest. In the first place, in our laboratory we have approximately 70 recently studied cases of retinitis pigmentosa, and every one of these, under certain conditions, can be found to have an extinguished electroretinogram. I think that every one of Dr. Goodman's patients can be found to have an extinguished electroretinogram, under certain conditions. This points up, I believe, the importance of standardization of the testing techniques. In one instance, that of a five-year-old child of a family of dominant retinitis pigmentosa, our findings were exactly the same as Dr. Goodman's inasmuch as we found a minimal potential. I think this is important, but I believe that this points up another factor: that children who have a perfectly normal function as far as we are able to determine, already show marked diminution in the amplitude of the electroretinogram. This extreme diminution means, to me, that there is an underlying mechanism beyond that of mere degeneration. There must be an alteration in the basic physiology of these children. By using brighter lights we have been able, in many instances, to produce some response, and this finding of Dr. Goodman's is a major contribution. I think that most of the cases that were originally reported were probably further advanced. I also think that possibly this case of Dr. Karpe's of the seven-month-old child must be very carefully examined. I have never had a similar experience, but there is normally a delay in the development of the electroretinogram in the immediate postnatal period. Immediately following birth there is no electroretinogram. Perhaps the visual units of this infant were such that the development of the electroretinogram was delayed. There is one more point I would like to discuss and that is the point of Nembutal sedation. This is part of the paper we are going to read this afternoon, but if I may, I would like to mention it.

A succinyl-choline paralyzed cat given successive doses of Nembutal will have an increased amplitude of electroretinographic response with increasing dosage of anesthetic. The a-wave increases more than the other components. Similar experiments on monkeys but with one optic nerve cut reveal that the electroretinographic responses become larger only in the eye with the intact optic nerve.

In our opinion, this signifies that there is a supraretinal control of the electroretinogram, which we shall discuss this afternoon. However, although dosages here are much larger than those used by Dr. Goodman for sedation, the sedation may play a part.

Fuortes: Thank you very much, Dr. Jacobson. We have now a comment from Dr. Brown.

Brown: I should like to suggest an alternative explanation as to why the electroretinogram disappears completely in retinitis pigmentosa.

It seems to be established that in certain

cases of retinitis pigmentosa the electroretinogram is almost completely absent while visual performance is almost normal. Since some components of the electroretinogram probably reflect essential steps in the visual process, it is difficult to understand such cases entirely as dysfunctions of processes which produce the electroretinogram. Riggs has offered an alternative suggestion that the abnormally small electroretinogram is due to perforations of the retina which short-circuit the electroretinogram. According to this interpretation, normal or near-normal electric activity might be produced but not recorded. Some recent findings make this suggestion even more plausible and indicate where the perforations would be most effective. Brindley has demonstrated with micropipette electrodes that most of the electroretinogram of the frog retina is measured across a high resistance membrane, which he designates the "R membrane" and tentatively identifies as the external limiting membrane. In the cat we can clearly distinguish the external limiting membrane from Bruch's membrane, using micropipette electrodes, and we find that Bruch's membrane has the properties of the high resistance membrane described by Brindley. Since most of the electroretinogram is measured across Bruch's membrane, the electroretinogram would be reduced by any serious damage to that membrane. Bruch's membrane would also seem in a particularly vulnerable position from the standpoint of damage by retinitis pigmentosa. Thus it would be interesting, in cases of retinitis pigmentosa, to see careful histology on Bruch's membrane in conjunction with electroretinogram and psychophysical measurements.

FUORTES: Thank you, Dr. Brown. We have three more questions. The first is from Dr. Sloan.

SLOAN: I'd just like to ask Dr. Goodman, first, is it not true that in every case in which he was able to do the psychophysical tests that there was abnormality in rod thresholds when there was an abnormal electroretinogram? If so, I don't think there is any mystery. Ordinary clinical tests do not include tests for rod vision. In the younger children, we can't use our light threshold tests. Dr. Goodman's tests can be given to three- and four-year-old children, whereas our psychophysical tests of rod vision have to wait until they are possibly eight years of age.

FUORTES: Thank you very much, Dr. Sloan. We have now Dr. Lipetz.

LIPETZ: At Columbus, we have been studying a family with retinitis pigmentosa, which is apparently carried through the females and expressed in the males. This is a very preliminary report. I will agree with Dr. Sloan that while in a number of these patients you can detect a slight change in the electroretinogram, especially during dark adaptation, you can also see such a change if you do the psychophysical adaptation study. Nevertheless, looking at the big changes that will occur in the electroretinogram records, as compared to normals and other retinal diseases, one does wonder if there is more than just a degeneration of the visual cells involved. Following the line of thinking of Dr. Jacobson and Dr. Brown, I would like to investigate that question further.

Now, I have some preliminary data which indicate to me that in these patients there is a change in the electric impedance of their eyes as compared with normal, and also a change when they change their state of adaptation. It may be that this change of the electric impedance is associated with a high-resistance membrane, let us say Bruch's membrane, of the eye. That's where we would expect to find a change in retinitis pigmentosa.

Histologic studies show that the first degeneration occurs in the neighborhood of the pigment epithelium. Noell produced an artificial degeneration which resembled retinitis pigmentosa in all of its clinical aspects by inhibition of the glycolytic activity of the pigment epithelium. This inhibition changes the metabolism in the eye and the ion transfers

across that particular tissue. It may be that this is the mechanism of retinitis pigmentosa, in which case we would expect to find a change in the electric properties of this tissue, and it is just such changes which I hope I will be able to detect in our further studies.

FUORTES: Thank you very much, Dr. Lipetz. We have now, Captain Wagner.

WAGNER: One point Dr. Lipetz mentioned in regard to degeneration in Bruch's membrane. It has been some years since I looked at this point, however, but aren't degenerative changes in Bruch's membrane a rather constant feature of tapetoretinal degenerations? The idea that a membrane such as Bruch's might be short-circuited was brought out, if I am not mistaken, by Loren Riggs some years ago. A paper has come out recently by Dr. Robert Cohn on the cat subjected to hypothermia. The electroretinogram disappeared, yet spike activity remains. Of course, we don't know that this means the cat is seeing anything, but we assume that since there are discharges from the nerve, there is optic nerve activity without an electroretinogram. However, they were quick to point out that certain other possibilities would have to be worked out before this report can be accepted.

FUORTES: Thank you very much, Dr. Wagner. Dr. Wald would like to say a few words now.

WALD: I have been looking for an opportunity to make some irrelevant remarks accompanied by a couple of slides I have in my pocket, and I would like to discuss disappearance and reappearance of the electroretinogram of the rat in vitamin-A deficiency. These are measurements made by my student, John Dowling, and will be published elsewhere (Dowling, J. E., and Wald, G.: Vitamin-A deficiency and night-blindness. Proc. Nat. Acad. Sci., **44**: in press, 1958). I think they illustrate a couple of points that have a bearing on what we are talking about.

Figure 14 shows results obtained in rats kept on a vitamin-A deficient diet for the number of weeks shown at the top of the figure. The percentages of rhodopsin extractable from the retina of these animals are shown on the second line. The dark-adapted threshold was measured by means of the electroretinogram, and the logarithm of the threshold is on the third line; the normal threshold is given the arbitrary value 1, that is, log threshold = 0. You see that the rhodopsin content begins to fall in about the fifth week of the deficiency; it falls rapidly thereafter, and the threshold correspondingly rises. The electroretinograms show a very interesting series of changes. You see each strip shows electroretinograms of two animals, and the log luminance used in a 50th of a second flash to stimulate them.

We begin at the left with a pair of normal animals; the animals remained normal through the fourth week of the deficiency, so we show one at the beginning of the diet and one in the fourth week. After that we have paired animals for each week of the deficiency. Now, I hope you can see that three things are happening.

On the one hand, as the threshold rises, you can no longer excite any electroretinogram with low luminances. By the eighth week, you see that we need a luminance three log units above what was sufficient to excite the normal retina in order to get an electroretinogram; that means a multiplication of our test stimulus by a thousand times, but if you do that you get your electroretinogram. This is, of course, the point emphasized by Dr. Goodman, that if you use a strong enough light you get an electroretinogram, when with a weaker light you don't.

The second thing is that the a-wave disappears much faster than the b-wave. The b-wave is declining as the deficiency progresses, but the interesting thing is that the a-wave is going much faster; so that in the last week of the deficiency at the highest luminance we still have a good b-wave but only a just perceptible a-wave. Yet the a-wave is very large to begin with. The a-wave goes out before the b-wave.

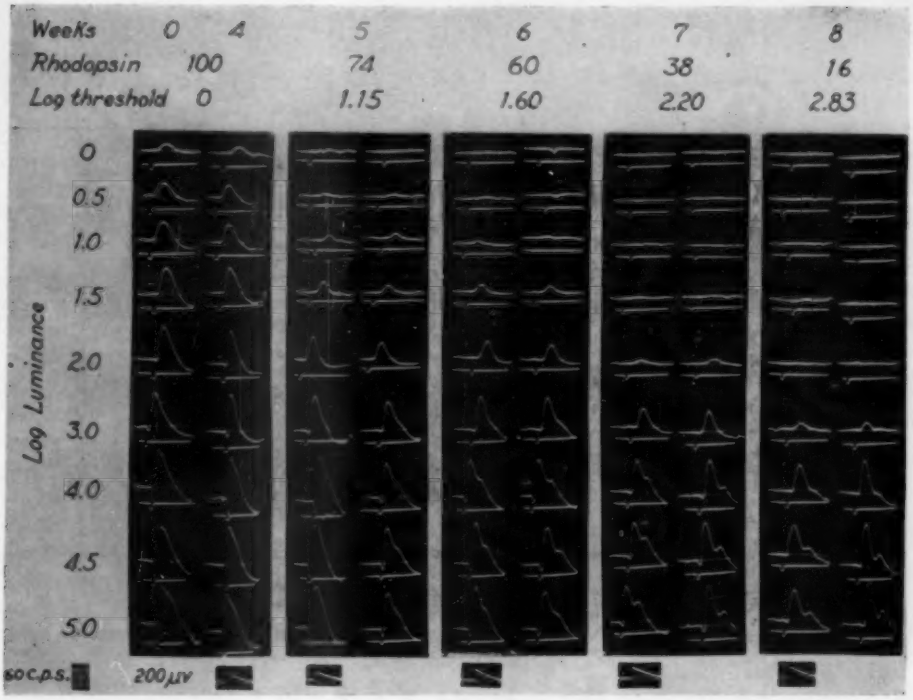

Fig. 14* (Goodman and Gunkel). Results obtained in rats on vitamin-A deficient diets. (From Dowling, J. E., and Wald, G.: Vitamin-A deficiency and night-blindness. Proc. Nat. Acad. Sci., **44**: in press, 1958.)

The third thing I would like to mention is the strangest of all. What appears in the normal animal as just a little inflection, especially at the highest intensities, on the shoulder of the b-wave, appears later and later as the deficiency progresses, and becomes entirely separated finally from the b-wave, appearing as a second positive component. Figure 15 shows what happens when you cure such animals. All you have to do is give them an intraperitoneal injection of vitamin A and, within the number of hours shown at the top of the figure, the threshold comes back to normal, so that 64 hours after the injection of vitamin A, this animal is completely cured of its night-blindness. If you look at the electroretinograms you see that they just reverse the changes that I just described to you. It is not only that the cure returns the electroretinogram to its normal size, but also its normal shape: the b-wave gets bigger, the delayed component once again merges with the b-wave, and the a-wave is re-instated.

The last point I would like to raise is that Dr. Goodman's paper emphasized that one portion of the a-wave or b-wave is due to the rods and another portion to the cones. The rat electroretinogram shows exactly similar doubleness in the a- and b-waves; yet rats are not supposed to have any cones. (Note added in press: Dr. Richard Sidman of the National Institutes of Health has since reported finding cones in the rat, about one cone to 10 rods.)

FUORTES: Thank you very much Dr. Wald. I am glad you decided to make these remarks because I couldn't think of any better contribution for closing this sympos-

* Figures 14 and 15 were presented by Dr. Wald.

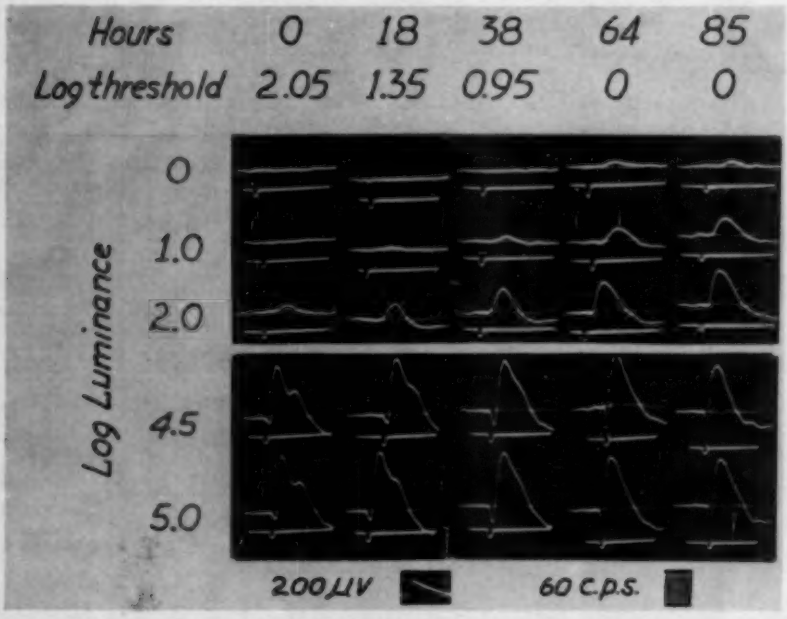

Fig. 15 (Goodman and Gunkel). Results obtained in vitamin-A deficient rats after intraperitoneal injections of vitamin A. (From Dowling, J. E., and Wald, G.: Vitamin-A deficiency and night-blindness. Proc. Nat. Acad. Sci., **44**: in press, 1958.)

ium. Dr. Goodman will now reply to the questions and comments.

GOODMAN: I think that there were two major questions asked. The first question, and one that has been asked from the very beginning of electroretinographic studies in retinitis pigmentosa, was: How could you have what appeared to be normal visual function and an absent electroretinogram? There is one thing that has to be stressed in answer to this, and Dr. Sloan has hit the nail right on the head. Even in the very early cases, the five-year-olds, where the electroretinogram was depressed, there was already definite evidence of marked rod dysfunction as shown by the two log-unit elevation of final light thresholds.

Now, if we will remember that most of the earlier studies of the electroretinogram in retinitis pigmentosa were done with weak light stimuli, and that a weak stimulus elicits an electroretinogram which reflects essentially rod function, it might be expected that with such stimuli the electroretinogram would be severly depressed or absent. Cone function, which is what tends to persist longer in retinitis pigmentosa, is concentrated in an electroretinographic complex which is elicited by higher stimulus intensities, and is much smaller in amplitude than a normal rod response.

A congenitally night-blind patient, such as the patient with Oguchi's disease, whose electroretinograms were demonstrated at the start of the talk, can have just a small a-wave and positive wave when dark adapted, yet within this small response is concentrated the whole of photopic vision with its normal visual fields under standard illumination, normal visual acuity, and photopic light thresholds. In such a patient, if you use a weak stimulus, you won't obtain any electroretinogram. If the stimulus is strong enough a response will be obtained

which looks markedly depressed in amplitude compared to the normal dark-adapted electroretinogram, but actually represents a normal cone response. These are some of the reasons why you can have fair photopic visual function in retinitis pigmentosa, while the dark-adapted electroretinogram is absent or markedly subnormal in amplitude.

Now, it certainly is possible that there are factors other than a simple degeneration of the rod and cone cells which account for the abnormal electroretinogram in retinitis pigmentosa. As a matter of fact, the second major question of the discussants dealt with the possibility that other factors are involved, in particular, changes in Bruch's membrane. A point was made that early in retinitis pigmentosa this membrane is affected. I don't think that this is borne out in histologic studies of retinitis pigmentosa.

Something many people have not paid too much attention to is the study of inherited retinal degenerations in animals which simulate human retinitis pigmentosa. And in this field it has been shown by Tansley (Acta Concil. Ophth., 3:1918-1926, 1954), Parry and others (Brit. J. Ophth., 39:349-352, 1955) that the early stages of the degeneration lodge in the rods and cones themselves. As a matter of fact, if one follows these affected Irish Setter dogs, one finds that at 22 days after birth there are degenerative changes in the rods. There is no electroretinogram as yet. This degeneration of the nuclei increases but, as it is increasing, some of the rod outer segments are developing. As they develop, the electroretinogram develops, but enough receptors have degenerated by about the 30th day so that there is no more electroretinogram. At this point there is no change in Bruch's membrane.

In humans these early histologic changes have not been studied but the examination of the early stages in these inherited degenerations in animals shows that the basic change is in the rods and cones—the rods first—not in Bruch's membrane. If changes occur at all in Bruch's membrane in these animals, or humans, and I don't know if they do, they occur much later.

A point was made about the use of different stimulus intensities. I fully agree that with strong stimuli you get electroretinograms which cannot be obtained with weaker stimuli. But I want to make one point clear, that though we had to use strong intensities to get responses the fact is that the electroretinograms obtained were not just "little wiggles" but were over 200 microvolts in some cases. Furthermore, this study even suggested that the electroretinogram might be normal in the very early stages of the disease. Certainly, we have seen that the electroretinographic cone response can be normal in affected children. We will have to follow the two youngest children who had normal dark-adapted electroretinograms as well to see if either of them go on to the disease.

We think that the electroretinogram is a less sensitive indicator of retinal dysfunction than subjective tests, but its importance lies in the fact that you can't use subjective tests in young children. It is a crude mass response, so that in cases of retinal detachment, with over one quarter of the retina detached, you can often still have an apparently normal electroretinogram. This reasoning also applied to the early stages of retinitis pigmentosa. Patient B III 8 definitely has abnormal cone function. We see it in his visual fields, and his retinal profile studies. But his cone electroretinogram is within normal limits, and is actually a high normal response. The probable explanation for the normal electroretinographic findings, and the abnormal subjective findings, is that we need a great deal of change in the retina to show up as an abnormality in the electroretinogram.

A question was raised about the effect of barbiturates on the electroretinogram. It was a good point. I can only say that we recorded electroretinograms on some normals with and without the light sedation we use, and there was no difference in the electroretinograms. I also refer you to a study, done in another laboratory, where electroretino-

graphic changes in cats could be shown only with very high barbiturate doses (Wohlzogen, F.: Ztschr. f. Biol., **108**:217-233, 1956.)

I found Dr. Wald's comment about the early disappearance of the a-wave in vitamin-A deficient rats very interesting. We know that in normal human electroretinograms, as the stimulus intensity is reduced, the first thing to disappear is the a-wave. The a-wave requires a higher stimulus intensity than the positive components to be elicited. However, in our cases of retinitis pigmentosa, the negative waves tended to persist even when the positive waves were markedly depressed. As a matter of fact, we have a subject who was originally reported as a congenital night-blind, but who has subsequently been shown probably to have an atypical retinitis pigmentosa. This subject has a completely normal a-wave, with both rod and cone components, but showed only the cone positive component. The normal appearance and behavior of the a-wave were shown in quantitative dark-adaptation studies, and its intensity function.

This study, which was done with Dr. Hans Bornschein (Goodman, G., and Bornschein, H.: Arch. Ophth., **58**:174-182, 1957) is especially interesting because of the work of Noell and the evidence presented by Dr. Gouras and Dr. Brown yesterday, which show that the a-wave and positive waves may arise from different levels in the retina. These investigators all agree that the a-wave comes from more distal structures, like the outer segments of the receptors, and the positive waves arise closer to the bipolar layer. We postulated that in this patient a lesion had occurred somewhere in between the two, let us say in the inner segments of the receptors, and which might allow production of a normal a-wave, yet block transmission to the structures generating the positive waves, and to the cortex. This would explain the appearance of the scotopic portion of the a-wave, but the absence of the b-wave, and the absence of normal rod thresholds on subjective tests. This case is also interesting because it shows that the electroretinogram may demonstrate differences in patients with night-blinding diseases which cannot be shown in subjective tests, and may be an aid in showing genetic heterogeneity in diseases which outwardly at least appear to be similar.

I agree with Dr. Wald that one must be cautious in labelling the electroretinogram waves as "rod" or "cone" waves. However, I deliberately perfaced this study by showing the electroretinograms obtained from a congenital night-blind (a case of Oguchi's disease), a total color-blind, and a normal subject, under the same conditions used in this study, in order to show that in the human, using certain techniques, one can convincingly separate rod and cone function. We could demonstrate that one could practically add the photopic response of the congenital night-blind to the scotopic response of the total color-blind, and end up with a normal electroretinogram.

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